



University
of Glasgow

<https://theses.gla.ac.uk/>

Theses Digitisation:

<https://www.gla.ac.uk/myglasgow/research/enlighten/theses/digitisation/>

This is a digitised version of the original print thesis.

Copyright and moral rights for this work are retained by the author

A copy can be downloaded for personal non-commercial research or study,
without prior permission or charge

This work cannot be reproduced or quoted extensively from without first
obtaining permission in writing from the author

The content must not be changed in any way or sold commercially in any
format or medium without the formal permission of the author

When referring to this work, full bibliographic details including the author,
title, awarding institution and date of the thesis must be given

Enlighten: Theses

<https://theses.gla.ac.uk/>
research-enlighten@glasgow.ac.uk

Life history patterns and reproduction in the threespine stickleback
(*Gasterosteus aculeatus* L.)

by

Angela Adanze Ukegbu

A thesis submitted for the degree of
Doctor of Philosophy

Department of Zoology
University of Glasgow

April 1986

ProQuest Number: 10991764

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10991764

Published by ProQuest LLC (2018). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

CONTENTS

	PAGE
ACKNOWLEDGEMENTS	13
ABSTRACT	14
CHAPTER 1 INTRODUCTION	17
1.1 Life History Theory	18
1.2 Introducing Behaviour Into Life History Theory	23
1.3 Life History Studies Of Teleost Fish	30
1.4 Age Structure Of Three Spine Stickleback Populations	33
1.5 Gonadal Development And Endocrinological Control In Sticklebacks	35
1.6 Reproductive Behaviour Of Sticklebacks	37
1.7 Adaptive Variation and Reproduction In Sticklebacks	37
1.8 Summary Of Aims	39
CHAPTER 2 GENERAL METHODS	40
2.1 Study Sites: Location and Description	41
2.2 Sampling Methods	41
2.3 Fish Care and Laboratory Conditions	42
2.4 Statistical Analysis	43

CHAPTER 3	AGE STRUCTURE OF THREE SCOTTISH STICKLEBACK POPULATIONS	44
3.1	Introduction	45
3.1.1	Techniques for ageing fish	46
3.1.2	Techniques applied to sticklebacks	48
3.2	Materials and Methods	51
3.2.1	Sampling regime	51
3.2.2	Preparation and interpretation of otoliths	52
3.2.3	Assigning fish to age classes	52
3.2.4	Breeding in early and late hatched fish	53
3.2.5	Data analysis	53
3.3	Results	54
3.3.1	Growth of otoliths	54
3.3.2	Typical difficulties encountered in interpreting otoliths	60
3.3.3	Pattern of ring formation	63
3.3.4	Length frequency distribution	69
3.3.5	Population age structure	73
3.3.6	Breeding in early and late hatched male sticklebacks	73
3.4	Discussion	73
CHAPTER 4	FEEDING INTENSITY AND DIET COMPOSITION	77
4.1	Introduction	78
4.2	Materials and Methods	82
4.2.1	Stomach analysis	82
4.2.2	Data analysis	84

4.3	Results	87
4.3.1	Stomach fullness	87
4.3.2	Percentage of fish with empty stomachs	97
4.3.3	Diet composition	102
4.4	Discussion	108
4.4.1	Stomach fullness	108
4.4.2	Diet composition	110
CHAPTER 5	GONADAL DEVELOPMENT AND ITS RELATIONSHIP TO LENGTH, WEIGHT AND FOOD INTAKE	112
5.1	Introduction	113
5.1.1	Somatic and gonadal growth in fish	113
5.1.2	Gonadal development in sticklebacks	114
5.2	Materials and Methods	117
5.2.1	Data analysis	126
5.3	Results	128
5.3.1	Seasonal changes in gonadal condition	128
5.3.2	Differences between populations	142
5.3.3	Gonad weight/body weight ratio and maturation score in fish of different age groups	144
5.3.4	Association between body features and gonadal development	149
5.3.5	Relationship between stomach fullness and morphological development	153
5.4	Discussion	156

CHAPTER 6	SEASONAL INFLUENCE ON RISK-TAKING DURING REPRODUCTION IN STICKLEBACKS	163
6.1	Introduction	164
6.2	Materials and Methods	167
6.2.1	Data analysis	169
6.3	Results	171
6.3.1	The effects of presentation of a predator	171
6.3.2	The effects of breeding early and late in the season	185
6.4	Discussion	189
CHAPTER 7	GENERAL DISCUSSION	194
REFERENCES		206

LIST OF TABLES

- Table 1.1 Pairwise-trade offs between N , S_1 , S_2 , T_1 and T_2 from Sibly and Calow (1983)
- Table 3.1 Frequency distribution of otolith types and mean and range of standard length of the fish in each category for River Kelvin
- Table 3.2 Frequency distribution of otolith types and mean and range of standard length of the fish in each category for River Luggie
- Table 3.3 Frequency distribution of otolith types and mean and range of standard length of the fish in each category for Aurs Burn
- Table 4.1 Months with similar percentage stomach fullness grouped for purposes of analysis
- Table 4.2 Summary of months for which percentage stomach fullness were significantly different: River Kelvin
- Table 4.3 Summary of months for which percentage stomach fullness were significantly different: River Luggie
- Table 4.4 Summary of months for which percentage stomach fullness were significantly different: Aurs Burn
- Table 4.5 Means and standard error of percentage stomach fullness for the populations showing those with significant differences
- Table 4.6 Mean and standard error of percentage fullness for the different sizes of fish from the River Kelvin
- Table 4.7 Mean and standard error of percentage fullness for the different sizes of fish from the River Luggie

Table 4.8	Mean and standard error of percentage fullness for the different sizes of fish from the Aurs Burn
Table 4.9	Sizes with significant differences in percentage fullness in River Kelvin
Table 4.10	Percentage of fish with empty stomach in the three populations
Table 4.11	Percentage composition of the diet at different times of the year in fish from the River Kelvin
Table 4.12	Percentage composition of the diet at different times of the year in fish from the River Luggie
Table 4.13	Percentage composition of the diet at different times of the year in fish from the Aurs Burn
Table 5.1	Mean and standard error of length, weight, gonad weight/bodyweight ratio, maturation score and condition factor for the three populations, showing those with significant differences
Table 5.2	Mean and standard error of gonad weight/body weight ratio and maturation score for females of different age groups of the 3 populations, showing those with significant differences
Table 5.3	Mean and standard error of gonad weight/body weight ratio and maturation score for males of different age groups of the 3 populations, showing those with significant differences
Table 5.4	Summary of results obtained from regressing gonad weight/body weight ratio and maturation score on length
Table 5.5	Summary of results obtained from regressing gonad weight/body weight ratio and maturation score on condition factor

- Table 5.6 Summary of results showing the relationship between percentage stomach fullness and different morphological features
- Table 6.1 Summary of minutes with significant differences for bites at tube
- Table 6.2 Summary of minutes with significant differences for time near tube
- Table 6.3 Summary of minutes with significant differences for time at partition
- Table 6.4 Summary of minutes with significant differences for duration of dorsal spine raising
- Table 6.5 A presence and absence table showing when only the egg males (cell III) or only the empty nest males went to the nest (cell II) as well as when both went (cell IV) or neither went (cell I)
- Table 6.6 A presence and absence table showing number of times when only the egg males (cell III) or only the empty nest males (cell II) hid in the weed as well as when both went (cell IV) or neither went (cell I)

LIST OF FIGURES

- Figure 3.1 Length frequency distribution for the months of January to December for fish from the River Kelvin.
- Figure 3.2 Length frequency distribution for the months of January to December for fish from the River Luggie.
- Figure 3.3 Length frequency distribution for the months of January to December for fish from the Aurs Burn.
- Figure 4.1 Percentage stomach fullness at different times of the year in male fish from the 3 study populations. (a) Kelvin (b) Luggie (c) Aurs Burn.
- Figure 4.2 Percentage stomach fullness at different times of the year in female fish from the 3 study populations. (a) Kelvin (b) Luggie (c) Aurs Burn
- Figure 5.1 Mean and standard error of gonad weight/body weight ratio against months for females of the 3 study populations. (a) Kelvin (b) Luggie (c) Aurs Burn.
- Figure 5.2 Mean and standard error of gonad weight/body weight ratio against months for males of the 3 study populations. (a) Kelvin (b) Luggie (c) Aurs Burn.
- Figure 5.3 Annual changes of percentage of the stages of gamete production in fish from the River Kelvin. (a) Females (b) Males.
- Figure 5.4 Annual changes of percentage of the stages of gamete production in fish from the River Luggie. (a) Females (b) Males
- Figure 5.5 Annual changes of percentage of the stages of gamete production in fish from the Aurs Burn. (a) Females

(b) Males.

- Figure 5.6 Mean and standard error of maturation score against months for females of the 3 populations. (a) Kelvin (b) Luggie (c) Aurs Burn.
- Figure 5.7 Mean and standard error of maturation score against months for males of the 3 populations. (a) Kelvin (b) Luggie (c) Aurs Burn
- Figure 5.8 Mean and standard error of condition factor against months for females of the 3 populations. (a) Kelvin (b) Luggie (c) Aurs Burn
- Figure 5.9 Mean and standard error of condition factor against months for males of the 3 populations. (a) Kelvin (b) Luggie (c) Aurs Burn
- Figure 6.1 Mean and standard error of the number of bites at the tube containing the stimulus male in each minute of the test for egg and empty nest males
- Figure 6.2 Mean and standard error of time spent near tube containing the stimulus male in each minute of the test for egg and empty nest males.
- Figure 6.3 Mean and standard error of time spent at partition in the 5 minutes of the test when the predators were present.
- Figure 6.4 Mean and standard error of duration of dorsal spines raised in each minute of the test for egg and empty nest males.
- Figure 6.5 Mean number of bites at the tube containing the stimulus male in fish tested in May, June and July for egg and empty nest males.

Figure 6.6 Mean time spent near the tube containing the stimulus male in fish tested in May, June and July for egg and empty nest males.

Figure 6.7 Mean time spent at partition in the 5 minutes of the test when the predators were present in fish tested in May, June and July for egg and empty nest males.

LIST OF PLATES

- Plate 3.1 Otolith assigned to category (CO), with a centre and one opaque ring
- Plate 3.2 Otolith assigned to category (COT), with a centre, one opaque ring and one transparent ring
- Plate 3.3 Otolith assigned to category (COTO), with a centre, two opaque rings and one transparent ring
- Plate 3.4 Otolith assigned to category (COTOT), with a centre, two opaque rings and two transparent rings
- Plate 3.5 Otolith assigned to category (COTOTO), with a centre, three opaque rings and two transparent rings
- Plate 3.6 An example of an otolith with no demarcations and which is uniformly opaque
- Plate 5.1 Section of the stickleback ovary showing the first four stages of ova development
- Plate 5.2 Section of the stickleback ovary showing stage five
- Plate 5.3 Section of the stickleback ovary showing stage six
- Plate 5.4 Section of the stickleback ovary showing collapsed follicles
- Plate 5.5 Section of the stickleback testis showing spermatogonia, spermatocytes and spermatids
- Plate 5.6 Section of the stickleback testis showing spermatozoa throughout
- Plate 5.7 Section of the stickleback testis showing a post spawning male with empty tubules

ACKNOWLEDGEMENTS

I am indebted to my supervisor, Dr Felicity-Ann Huntingford for her constant encouragement, enthusiasm and advice throughout the period of this project; she also went out of her way to make my first few months in Glasgow bearable and this will never be forgotten.

My sincere thanks also go out to the following people who have given invaluable help at various stages on the project: Mr Maclaren advised on the statistical methods used for the analysis of the data; Dr R. J. Wootton and Dr Nick Giles solved initial problems encountered with ageing sticklebacks using their otoliths; Dr David Scott gave practical help on the identification of the stages in testis development and Dr Spurway and Mr Rickus gave photographic assistance.

I owe a lot to my parents who single handedly financed my entire educational career. This study would not have been possible without them. Lastly, I thank Mickey Umeorah for amongst other things his continual support and understanding over many years which I have come to count on.

ABSTRACT

This thesis describes research on life history patterns and reproduction in the three spined stickleback (Gasterosteus aculeatus L.) from three Scottish populations. The populations are from Rivers Kelvin and Luggie and the Aurs Burn which are broadly similar habitats located in and around Glasgow.

Age structure of the populations was studied using length frequency distribution and otolith analysis and both methods showed the life span of the fish to be just over a year, with a few females of the River Kelvin and Aurs Burn surviving to two years. Although the patterns of length frequency distribution of the three populations were generally the same, Kelvin fish were longer and heavier compared to fish from the other two populations.

Most sticklebacks from these populations reproduce in the breeding season of the year following hatching when they are about a year old. However in all three populations, some fail to reproduce at this age. There is a protracted breeding season from April to August and there is evidence to suggest that those fish that do not breed at one year are offspring bred at the end of the previous season. They only reach maturity when the breeding season is almost over and therefore are not able to reproduce. The chances of this group of fish surviving to breed in the subsequent year at age two plus are very slim. Thus in these populations, there is a category of fish hatched late in the season which is extremely unlikely to breed at any stage of life.

Monitoring the food intake of the sticklebacks showed that stomach fullness levels were low in winter and high just prior to the breeding season. No differences in food intake were found between the sexes or between age classes. Kelvin and Luggie fish of both sexes were however found to be consuming more food than the Aurs Burn fish.

Quantitative histological examination of the gonads showed that the more mature stages of the ova were predominant in the females of all 3 populations during the breeding season, as would be expected. In the males, high levels of spermatozoa appeared at different times in the 3 populations. High levels could be seen in the Aurs Burn males as early as December but not until much later in males of the other two populations.

Older and larger females had relatively heavier gonads and higher maturation scores and in Kelvin and Aurs Burn fish this was positively related to body condition. No relationship was found between age and relative gonadal weight in males, although older fish had more mature gonads. In one year old males from the Kelvin and Aurs Burn, a negative relationship was found between gonadal development and body condition.

In fish from the Rivers Kelvin and Luggie, there was no relationship between percentage stomach fullness and body condition, suggesting that food is not limiting for these fish. However, Aurs Burn fish had a positive relationship between percentage stomach fullness and body condition. They also had the lowest food intake suggesting that food is in short supply. These fish appeared to be investing in

growth and gonadal development at the expense of body condition.

Since late hatched broods have little chance of breeding and so are less valuable, experiments were carried out at different stages of the breeding season to investigate allocation of time between nesting, territorial behaviour and self defense of parental males when at a risk of predation. These showed that male sticklebacks take risks to defend their young and that the intensity of this defense is greater in the presence of a brood. The presence of a predator affects this response; while males with eggs were attacking the conspecific intruder (placed near the nest) and visiting their nests more, males with empty nest tended to hide in the weed or investigate the predator. Males breeding early in the season showed a higher degree of parental investment compared to those breeding late in the season. This last result was attributed to the higher value of broods at the beginning of the breeding season. However, it remains enigmatic, since in these populations at least, the males have an almost non-existent chance of surviving to breed again in the next year.

CHAPTER 1
INTRODUCTION

1.1 LIFE HISTORY THEORY

" Natural selection favours those individuals who most abundantly transmit copies of their genes to future generations" (Horn and Rubenstein 1984). Among the many features of living organisms which may influence their success in transmitting copies of their genes are those concerning the allocation of resources to reproduction.

Most organisms have only limited access to resources necessary for building and maintaining their own bodies as well as forming new individuals. Any resources used in one of these necessities will not be available for the others and the amount invested in these various processes (growth and reproduction) is likely to be a critical determinant of fitness. The scientific analysis of this aspect of resource allocation is usually called life history theory and it seeks to understand the selective forces that mould life history characteristics such as growth, lifespan, age of maturity and fecundity. Comparison between and within groups of a wide range of animal species have provided important information on the adaptiveness of life history parameters. However a number of selective forces are involved which interact to create a complex system best handled by means of theoretical models.

In this context a model is an attempt to represent some or all the essential features of an animal's life history characteristics (such as rate of reproduction, survival and growth) and the relationships between them in mathematical terms which ideally are kept as simple as possible. From this, the outcome of interactions between life history variables in terms of rate of population growth can be

calculated, and predictions made and tested. If a model accurately predicts the distribution of life history characteristics in real animals, then the theory about the interactive outcome of these features that it embodies is supported.

Most life history models have used optimization theory to examine the selective forces acting on reproductive strategies. This theory states that for any investment made by an organism, there is a return in fitness; a cost-benefit function arises which can then be solved to show the level of investment that maximizes the net rate of return in fitness. Several biological decisions are important for the animal: for example, what is the optimum number of times to reproduce and the optimum number of young per brood? At what age should reproduction commence and should a few large or many small young be produced? Life history theory tries to predict the best decisions (that is, those which maximizes relative fitness gain) to make in the face of the problems posed by the different environments; many studies have been carried out to test these predictions.

The value of life history models can be demonstrated by a consideration of the interaction between litter size and frequency of reproduction. Charnov and Schaffer (1973) extended an earlier model of Cole (1954) and Bryant (1971) by considering variations in both adult and juvenile mortality rates. They compared an annual and perennial species that both bred at the end of their first year. Members of the annual species die after producing B_a offspring per litter. The perennial species produces B_p offspring per individual but lives to reproduce in subsequent years. For both species, the proportion of offspring surviving their first year is C while the

adult survival rate of the perennials is P. The relationship for the rate of population growth (R) for the two populations will be given by

$$N(T+1) = R \cdot N(T)$$

where $N(T)$ is the number of organisms in year T just before reproduction and after the years mortality and $N(T+1)$ is the number of organisms in the subsequent year. Thus for the annual species,

$$N(T+1) = B_a \cdot C \cdot N(T)$$

Therefore

$$R_a = B_a \cdot C$$

The growth rate for the perennial species is equivalent to the annual species except that it includes the contribution due to the survival of the previous breeders ($P \cdot N(T)$). For the perennial species therefore,

$$N(T+1) = B_p \cdot C \cdot N(T) + P \cdot N(T) = N(T) (B_p \cdot C + P)$$

Therefore

$$R_p = B_p \cdot C + P$$

If the annual and the perennial populations are to increase at the same rate,

$$B_a \cdot C = B_p \cdot C + P \text{ i.e. } R_a = R_p, B_a = B_p + (P/C)$$

In both Cole's and Bryant's models,

$$P = C \text{ giving } B_a = B_p + 1 \text{ which is Cole's result.}$$

Therefore Charnov and Schaffer restated Cole's result to say that an annual organism could become reproductively equivalent to a perennial by adding P/C individuals to their average litter size. What these models have ignored is the complexity of sexual reproduction. Waller and Green (1981) extended the model to sexual organisms and concluded that a sexually reproducing animal that bears $B + 2P/C$ young and then dies is reproductively equivalent to a perennial that bears B young each year for as long as it lives where P = adult survival rate till next breeding season and C = number of young that survive their first year. This addition $2P/C$ is less costly if litter size is large and offspring are small and need little parental care. The value of $2P/C$ is also lowered by low parental survival and high offspring survival.

Using a similar approach, Horn and Rubenstein (1984), and indeed several earlier authors, have come to the conclusion that such factors as large litters, small offspring, little parental care, low parental survival and high survival of juveniles favour early and exhaustive breeding. Conversely, small litter, large young, parental care, high parental survival and low survival of young are the factors that favour restrained early breeding.

In most life history models, the importance of age at maturity has been emphasized (for example, Stearns 1976; 1980; Waller and Green 1981). In order to understand the selective forces acting on rates of maturity, the advantages and disadvantages of early and late

breeding and how this depends on environmental circumstances need to be identified. One of the advantages of early breeding is that the offspring of a young parent might already be breeding by the next time their parent breed thus multiplying their reproductive benefit. Horn and Rubenstein (cited above) state that when early breeding is favoured, it is more highly favoured in species with rapid development to maturity. Late and exhaustive breeding has been shown to be adaptive in environments where predators of adults are rare and with high juvenile mortality. Reznick and Endler (1982) found that guppies in Trinidad mature and breed early in streams where predators of adults are common and breed later where they are rare.

Glebe and Leggett (1981) found that shad populations in Florida rivers breed exhaustively where juvenile survival is high; in rivers where conditions are less predictable however spawning occurs in several more conservative episodes. Schaffer and Elson (1975) gave the same reasons for the exhaustive spawning of Pacific salmon (Oncorhynchus) in comparison to the more repeated spawning of the Atlantic Salmon (Salmo Salar). The young of the latter species face the unpredictable dangers of melting ice floating down the streams in spring.

Juvenile and parental mortality has therefore been shown to have opposite effects on adaptive patterns of breeding. Juvenile mortality biases parents towards conserving resources and investing them in themselves or a few large young while adult mortality has the effect of making parents invest in many small young even if this poses a threat to their own life.

In 1984, Gross explained that the use of optimization theory in understanding reproductive tactics has a shortcoming in that it does not take into account the fact that the optimal tactic to adopt by animals might depend on what others are doing. Games theory incorporates such frequency dependent components and could therefore be used to analyse this problem. The theory states that "if fitness payoffs depend both on the strategy adopted by an individual and on those employed by other members of the population, the strategy which evolves need not be optimal but instead must be an evolutionarily stable strategy (ESS)" (Maynard Smith 1982). The ESS is defined as "a strategy which when adopted by a critical proportion of the population, yields an individual fitness which exceeds that of all alternative strategies when those are rare in the population". Gross (cited above) used this approach to analyse the occurrence of precocious sexual maturity in salmon and sunfish (see below).

1.2 INTRODUCING BEHAVIOUR INTO LIFE HISTORY THEORY

A factor that obviously influences adult and juvenile survival is the degree of parental care that a species shows. While parental care enhances juvenile survival, it usually decreases parental survival. In seasonally breeding species there will often be cases when an adult gets a better pay off by investing in an additional brood in the present breeding season (thus decreasing the chances of surviving to breed again) rather than waiting for an uncertain future breeding episode (Hirshfield and Tinkle 1975).

In the theoretical analyses of life history characteristics, the initial simple models of Cole (cited above) and others have become

progressively more complex with such factors as age of maturity, size of young, parental care and cost of reproduction being relevant to population growth. Sibly and Calow (1983) have incorporated many of these parameters into a single model and thus are able to analyse trade-offs (how alterations in one parameter forces changes in others) between different life history parameters. Several of the parameters they consider have a behavioural component.

This model defines fitness as the intrinsic rate of increase of phenotypes and modifies the Euler-Lotka equation to include five different life history parameters:

1. The survivorship of the offspring from birth until first breeding (S_1).
2. The survivorship of the adult between successive breedings (S_2).
3. The number of female offspring produced per female breeding attempt (N).
4. Age of first breeding (t_1).
5. Interval between successive breedings (t_2).

Interactions occur between these variables and any possible trade-offs between any two are worked out to determine the optimum phenotype.

From the Euler - Lotka equation,

$$1 = \sum_t e^{-rt} L_t(P,E) N_t(P,E) \quad (1)$$

where $L_t(P,E)$ is the survivorship to age t of phenotype P in environment E and $N_t(P,E)$ is the number of female offspring produced

per breeding female of age t with phenotype P in environment E . In this version of the Euler-Lotka equation, r is the intrinsic rate of change of a particular phenotype. The above equation is simplified by assuming that age and experience does not affect the reproduction and survival rates of an adult. The number of offspring produced per each breeding attempt is always equal to N and $L_t = S_1 S_2^{(t-t_1/t_2)}$ where S_1 = survivorship from birth until first breeding at t_1 ; S_2 is the survivorship of the adult between successive breedings assumed to occur at intervals t_2 apart. Equation 1 then becomes

$$1 = e^{-rt_1} S_1 N + e^{-rt_2} S_2 \quad (2)$$

This equation relates fitness r to the five life cycle variables S_1 , S_2 , N , T_1 and T_2 . Since offspring survival until first breeding S_1 generally decreases with age at first breeding t_1 , the model assumes that $S_1 = e^{-u_1 t_1}$ where u_1 = chance of offspring dying per unit time. Similarly adult survivorship between successive breedings S_2 decreases with interval between successive breedings t_2 , it is thus assumed that $S_2 = e^{-u_2 t_2}$ where u_2 is the chance of the adult dying per unit time. In any analysis of t_1 and t_2 , u_1 and u_2 can be held constant respectively while S_1 and S_2 (not being generally independent of t_1 and t_2) cannot. If u_1 and u_2 are held constant equation 2 becomes

$$1 = e^{-(r + u_1)t_1} N + e^{-(r + u_2)t_2} \quad (3)$$

In any analysis involving t_1 and t_2 , the model uses equation 2 in the form of equation 3. Trade-offs between pairs of life history

parameters can now be considered with the supposition that the other variables are fixed at values assumed optimal as shown in Table 1.1.

Considering the three parameters that do not include time i.e S_1 , S_2 and N and assuming an animal with an annual cycle which breeds once a year ($t_1=t_2=1$), equation 2 becomes

$$e^r = S_1 N + S_2$$
$$r = \log_e (S_1 N + S_2)$$

Taking a particular fixed value of S_1 , r can now be calculated for various values of N and S_2 . A three dimensional graph of r against S_2 and N can thus be plotted and then compressed into a 2 dimensional form with points of equal fitness (r) falling on lines like contour lines on a map (Fig. 1.1). The next step in the process of determining optimum phenotype is to draw a trade-off curve that depicts the relationship between S_2 and N . Without previous information on the nature of this curve, the model assumes that S_2 is a negatively accelerating function of N . The optimum phenotype with respect to S_2 and N at that value of S_1 is then found graphically by superimposing the fitness landscape onto the trade-off curve (Fig. 1.2). The point on the trade-off curve that contacts the highest fitness line is the optimum phenotype. Repeating this procedure for different fixed values of S_1 allows for investigation on how optimum phenotype depends on juvenile survival; for example the model predicts that where juvenile survival is high, N should be large. This is a clear prediction from the model which can be tested with real animals. In this way, the interactions between any pair of life history parameters used could be plotted out and the optimal trade-

off point determined. Table 1.1 shows some of the trade-offs discussed in this model which are relevant to studies of reproductive behaviour.

Thus models can be used to study the complex interactions between life history parameters and have been extended to incorporate a number of factors affecting the behaviour of the animal being studied. However as compared to the sophistication of existing theory, there is relative lack of empirical data. All the behavioural components outlined in table 1.1 potentially apply to the behaviour of an intensively studied species of a teleost fish (Gasterosteus aculeatus). For example, males compete for breeding territories and rear young at costs in terms of energy expenditure and predation risk (Whoriskey and FitzGerald 1985a). The major aim of the project described in this thesis was to collect data on life history parameters of three populations of this fish and to relate this to their reproductive behaviour.

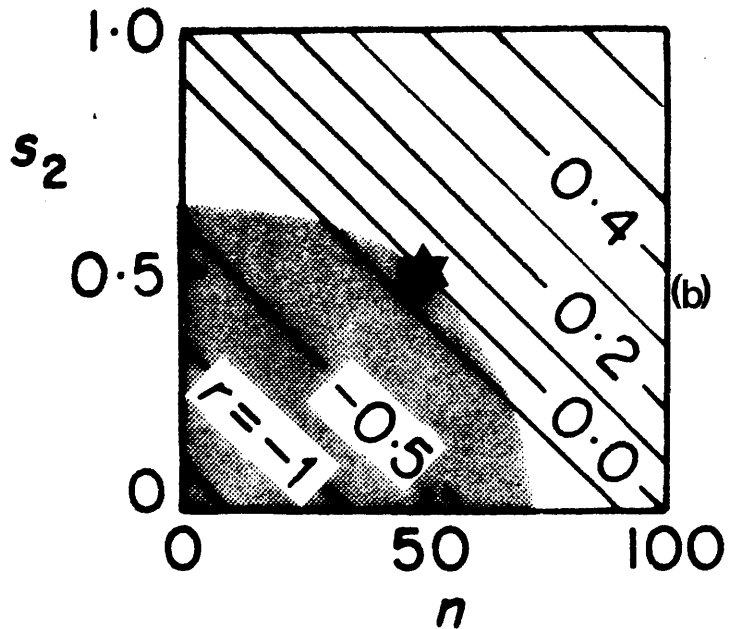
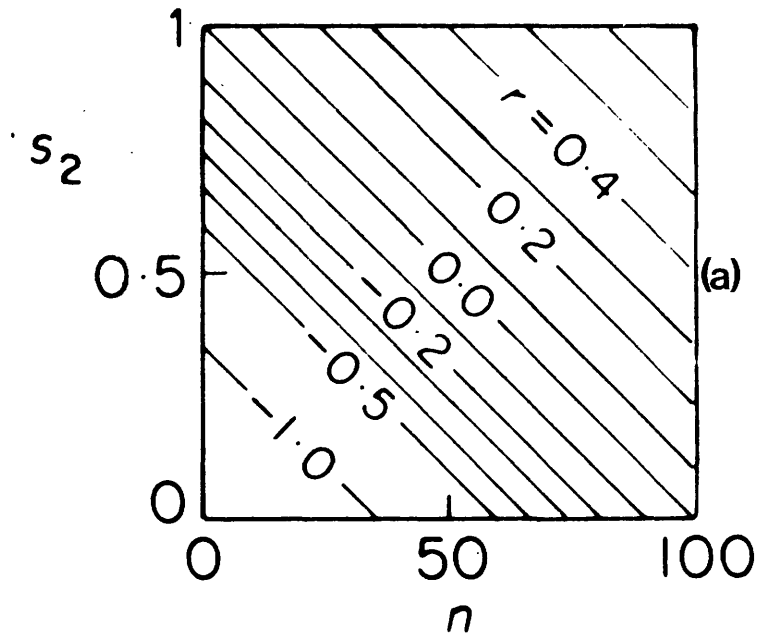


Fig. 1.1. (a) Fitness (r) plotted as a function of adult survivorship between breeding seasons (s_2) and number of offspring per female per breeding season (N).

(b) As in (a), with a hypothetical trade-off curve for s_2 and N ; * = optimum phenotype where survivorship of offspring between successive breedings $s_1 = .01$.

(From Sibly and Calow (1983))

Table 1.1 Pairwise trade offs between N , S_1 , S_2 , T_1 and T_2 .

Trade off	Interpretation
N vs S_2	Animals fighting for mates are increasing N and decreasing S_2
S_2 vs S_1	Parents taking risks to protect their brood are decreasing S_2 and increasing S_1 .
S_1 vs T_1	Young animals taking risks to acquire enough food to reach maturity, are decreasing both T_1 and S_1 .
T_2 vs S_1	Parents investing resources to protect its young are increasing both T_2 and S_1 .

1.3 LIFE HISTORY STUDIES OF TELEOST FISH

A distinctive feature of fish is the wide variation (both within and between species) in their life history parameters. Many attempts have been made to interpret these variations as an adaptation to the environmental conditions in which the fish live (Balon 1975). Life history theory suggests that the predictability of the environment is important.

Lambert and Ware (1984) compared the reproductive strategies of a demersal spawner - the herring (Clupea harengus) and a pelagic spawner - the Atlantic mackerel (Scomber scombrus). These two fish species have two extremes of spawning tactic and inhabit different environments. The mackerel spawns once in the summer and floods the environment with large batches of eggs during the time zooplankton production is highest. This allows a high growth rate for the larva which soon passes the critical size below which predators can get them. In contrast, the herring spawns in the autumn when food supply is poor but releases eggs in batches throughout the season thus spreading the risk of dying and chances of juvenile survival over a variety of environmental conditions.

Within the same species of fish however, local stocks may differ from each other in their life history characteristics in response to local conditions. Leggett and Carscadden (1978) studied the effects of latitudinal variation in reproductive characteristics of American shad (Alosa sapidissima). They proposed that the adult postspawning survival and fecundity represent different emphasis placed on reproduction and adult survival. The southern rivers in their study

were predictably good and the populations were semelparous (spawn only once) while the northern rivers were the opposite and had iteroparous (repeated spawning) populations. They therefore concluded that the "differences in the predictability of the reproductive environment through its influence on juvenile mortality was the principal regulator of latitudinal differences in life history strategies in shad".

Constantz (1979), studied the energy allocations of the Gila topminnow (Poeciliopsis occidentalis) in two contrasting environments - a spring run of constant characteristics and a fluctuating desert wash. The constant environment had both short and long lived fish while the fluctuating environment had only the short lived fish. When faced with food shortage, the long lived spring fish allocated less energy to both growth and reproduction while the short lived spring fish and the desert wash fish reduced only their energy allocation to growth.

Baltz and Moyle (1982) did comparative studies of life history traits within and between isolated populations of the tule perch (Hysterocarpus traski), each living under different environmental conditions. In a more predictable environment, females attain a large size but produce small broods at first reproduction. In a less predictable environment however, where longevity is shorter, females are smaller at first reproduction yet produce larger broods.

Baltz (1984) studied variations among female surfperches of the family Embiotocidae and showed how different environmental factors

had a strong effect on the evolution of their life histories. The females of three species of this family can be divided into three sizes. Adults of the largest species escape predation because of their size and therefore occupy open habitat; the smallest species stay only in habitats that provide complex cover and the medium sized ones are intermediate in their need. These environmental differences are proposed as being responsible for their different life histories by "mediating the availability of cover and risk of predation". The largest species have high fecundity, delayed maturity (for one to four years) and long life relative to the smaller species. The medium sized ones have low fecundity, can delay their maturity for one to three years and have intermediate life spans. Small species in general have a high fecundity but which is variable (depending on environmental predictability), do not delay maturity and are short lived.

The relative importance of different life history parameters as determinants of fitness vary between species. Jensen (1985) studied the relationship among net reproductive rate and life history parameters for lake whitefish (Coregonus clupeaformis). He concluded that changes in age at maturity and survival of immature fish are the only life history parameters that have a large effect on the net reproductive rate of the fish.

As stated above, Gross in 1984 showed how competition among individuals can result in alternative reproductive strategies which are commonly reported for fish conspecifics and how each of the alternatives could be evolutionarily stable. By using game theoretical analysis, he showed how the existence of alternative life

history tactics in north American sunfishes and the pacific salmon could be evolutionarily stable. These two fish species have parental males who mature at a large size but they also have males which adopt precocious development; called cuckolders in sunfishes and jacks in the salmon. They use alternative behaviour tactics to achieve mating success (for example, sneaking into the nest of the parental male to fertilize eggs). Gross showed how it is theoretically possible for these precocial males to achieve a fitness equal to that of the late maturing parental males, because although the benefits in terms of fertilised eggs may be relatively low, so are the costs of achieving this.

Nine spined sticklebacks (Pungitius pungitius) provide one of the earliest reported examples of alternative reproductive strategies, although the significance was not realised at the time (Morris 1970). Van denAssem (1967) also reported how some male three spined sticklebacks who had previously taken part in courtship competition and lost suddenly "freeze", lose their bright nuptial colours and adopt a cryptic pattern with dark bands across the back much like the females. They then attempt to sneak into the nest of the rival male to steal fertilization of the eggs.

1.4 AGE STRUCTURE OF THREE SPINE STICKLEBACK POPULATIONS

On the basis of size distribution, otolith studies and in some cases laboratory based growth studies, it is thought that three spined sticklebacks from many wild populations live for at least two full years, becoming sexually mature in their first year of life and then breeding in subsequent years (Mann 1971; Kimura et al 1979). In

contrast sticklebacks from a number of sites in Scotland appear to complete all their growth and reproduction within one year of hatching (Giles 1981). However most of these studies are usually based on rather small samples taken at few times of the year. An exception is a study involving extensive sampling over a year by Allen and Wootton (1982) who showed that sticklebacks in lake Frongoch are also annual. Thus existing data on age structure is relatively sparse and what is available indicates considerable variability. One aim of the present study was to characterize in detail the age structure of a number of geographically localised and ecologically similar populations, to investigate the extent of this variability.

Life history theory predicts that such variability might be related to environmental factors such as food levels and predation which determine the relative survival of adult and young sticklebacks. McPhail (1977) studied size at first maturity of female sticklebacks in 25 Southern Vancouver island populations and concluded that interpopulation differences in minimum size at first reproduction were inherited and probably represent local adaptations. He suggested predation by trout as a mechanism selecting for either small or large size at first reproduction; for example, where large predators are rare, selection may favour delaying maturity until fish become too large to be taken by small predators. When the reverse is the case, breeding as close as possible to the physiologically minimum size may be the optimal reproductive strategy (when fish are still too small to be taken by large predators). Mayer lake sticklebacks studied by Moodie (1972) have the largest minimum size at first reproduction (69mm) of any known population and Moodie also

suggests that the large size is at least in part an adaptation to escape predation since body size affects manipulation time and capture success of the trout predator.

In previous studies, systematic variation in population age structure has not been related to variation in patterns of gonadal development although theory predicts that these will be related. This thesis examines life history parameters in a number of stickleback populations with similar levels of predation to see if these can be related to food supply. An additional aim of comparing non-predated with heavily predated populations had to be abandoned due to unforeseen circumstances.

1.5 GONADAL DEVELOPMENT AND ENDOCRINOLOGICAL CONTROL IN STICKLEBACKS

The gonads of sticklebacks lie in the abdominal cavity, paired ovaries in the female and paired testis in the male. Detailed account of the undifferentiated germ cells and their subsequent sexual differentiation has been given by Swarup (1958). In his 1976 review, Wootton (citing Swarup 1958) reported that sticklebacks less than 9mm long have gonads that cannot yet be characterized as male or female and that the first maturation division is seen in the testis of a 25mm fish. At this stage they become fully differentiated containing most of the stages in the maturation of the sperm. The males do not however become functionally mature and able to carry out breeding until they are 40mm long. The general pattern of maturation is from spermatogonia - primary spermatocytes - secondary spermatocytes - spermatids - mature spermatozoa. The ovaries of the females are fused together at the caudal end where the

oviduct begins (Swarup 1958); Tromp-Blom 1959). The germ cells or oogonia divide to give rise to oocytes which then enlarge and finally mature to a ripe ovum.

Although a number of studies on gonadal development in sticklebacks have been carried out (for example, Craig-Bennett 1931; Swarup 1958) these have all been descriptive rather than quantitative. They also all indicated variability, particularly in the timing of maturation events. One aim of this present study is to collect quantitative data on gonadal development in sticklebacks from Scottish populations for comparative purposes.

Baggerman's (1957) study of G. aculeatus showed that sexual maturity in larger fish could be induced at any time of the year by appropriate manipulation of temperature and photoperiod. Craig-Bennett (1931) found no such influence, but both found that a high temperature favours gonadal development. It is assumed that long photoperiod induces the secretion of the gonadotropins of the pituitary and Ahsan and Hoar (1963) showed the gonadotropin-luteinizing hormone stimulates gonad development in fish maintained under the short photoperiod. The appearance of the male nuptial colouration and commencement of breeding behaviour has been correlated with evidence of increased steroidogenesis (Gottfried and Van Mullen 1967). They estimated that dominant males which have acquired territories have five to seven times the steroid levels of non-breeding males caught at the same time.

1.6 REPRODUCTIVE BEHAVIOUR OF STICKLEBACKS

The reproductive behaviour of sticklebacks has received more attention than any other aspect of the biology of this fish. The breeding season lasts for three to four months from spring to summer and the non reproductive period lasts for the rest of the year. During this non reproductive period, there are very few differences between males and females either in their morphology or behaviour. In the breeding season, the males take on their breeding colouration (the throat becomes red, and the irises of the eye become greenish blue) and start to defend territories. Within this territory, the males build nests constructed out of sand grains and vegetation glued together with a mucous secretion of the kidney. They then court gravid females who spawn their eggs in the nest and the male releases his sperm over the eggs to get them fertilized. With eggs in his nest, the male assumes a parental role and ventilates the eggs by fanning a stream of fresh water through the nest with his pectoral fins. When the eggs hatch, the male tears the nest to pieces, retrieves any of the young that stray away until they are more active and disperse. A few days after the young disperse, the male may build another nest and it has been estimated they can build up to five nests in a breeding season (Wootton 1976). The females also produce several clutches of eggs during the breeding season, the number depending on the size of the female and on her food supply (Wootton 1974).

1.7 ADAPTIVE VARIATION AND REPRODUCTION IN STICKLEBACKS

Theory suggests that the degree of investment in reproduction

will reflect complex costs and benefits and therefore could be expected to differ in fish with different life histories. As far as benefits are concerned, Van den Assem (1967) and Kynard (1978a) have shown that territorial aggression enhances reproductive success; thus aggressive males with large territories attract more females and are more successful in rearing their young. However, the complex reproductive behaviour of male sticklebacks has its costs (being both time and energy consuming), in particular potentially renders them vulnerable to predation (Whoriskey and FitzGerald 1985a).

Sticklebacks are prey to a number of species. The main predators of adult sticklebacks are fish eating birds, mammals and large carnivorous fish. Because of their relatively small size, eggs and larva have a wider range of predators. Predation, especially by other species of fish has probably been one of the most important selective forces shaping the evolution of the three spined sticklebacks (Wootton 1976). Sticklebacks have a number of adaptations to protect them against these predators. Both the dorsal and ventral spines can be locked in an erect position and provide protection against predators (Hoogland et al 1957). There are also behavioural adaptations such as vigilance and hiding under cover. Huntingford (1982) and Giles and Huntingford (1984) have shown that behaviour relative to parental investment (i.e territorial aggression) varies from site to site possibly in part as a result of local predation condition. One aim of this study was to look at the trade-off between self defense in breeding males and defense of brood in more detail. This could perhaps give some insight into the kind of selective forces that mould breeding behaviour in sticklebacks.

1.8 SUMMARY OF AIMS

Specifically the objectives of this study are:

- (1) To characterize age structure of sticklebacks in three broadly similar habitats in order to investigate how constant or variable age structure is.
- (2) To monitor food supply of sticklebacks in these populations and relate food intake of fish of different age and gender to life history parameters.
- (3) To carry out detailed quantitative examination of the annual gonadal cycle of the populations and to relate gonadal development to body growth and condition.
- (4) To use life history parameters to make predictions about the expected parental behaviour of male sticklebacks and particularly the risk of predation that they incur.

CHAPTER 2
GENERAL METHODS

2.1 STUDY SITES: LOCATION AND DESCRIPTION

The three study sites; Rivers Kelvin (4 degrees 18 minutes west longitude and 53 degrees 0 minutes north latitude), Luggie (4 degrees 10 minutes west longitude and 55 degrees 57 minutes north latitude) and the Aurs Burn (4 degrees 20 minutes west longitude and 55 degrees 48 minutes north latitude) are located in and around Glasgow. The River Kelvin is an extensive and fast flowing urban stream. Most of the sticklebacks were however caught from a slow flowing outflow of the river. The other fish species present were mainly minnows (Phoxinus phoxinus) and very few nine spined sticklebacks (Pungitius pungitius). The section of River Luggie that was fished lies immediately above the confluence of River Kelvin at Kirkintilloch. Although this was quite far from the section of the River Kelvin that was fished (approximately 10.7 km), it is not impossible that fish from the two study sites could mix. Fish species present were three spined sticklebacks, minnows, stone loach (Noemacheilus barbatulus), eels (Anguilla anguilla) and brown trout (Salmo trutta). The third stream studied is part of the River Cart system called the Aurs Burn situated at the outskirts of Glasgow. It is a shallow, fast flowing stream providing little plant cover for sticklebacks which make use of overhanging bushes for cover. Compared to the other two populations, it has the lowest density of sticklebacks but fish were abundant at all 3 sites. Other fish species were minnows, stone loach and small brown trout.

2.2 SAMPLING METHODS

A 40 cm diameter hand net was used to catch all the sticklebacks for behavioural experiments (during the breeding season) and for

morphological studies. The sticklebacks were normally found where the river was not fast flowing and usually hid underneath trees and aquatic vegetation. The fish were transported back to the laboratory in opaque plastic containers. All morphological samples were killed in the laboratory with MS 222 and after measurements and dissection, preserved in 95% ethanol, as formalin solution dissolves the inner ear bones (otoliths) used for ageing the fish.

To avoid depletion of the population with frequent sampling, it was necessary to find the minimum sample size which gives an accurate picture of the average length and weight of fish in the population. A plot of the mean and variance of length and weight of different sample sizes showed that with a sample size of twenty fish, the estimates stabilise.

2.3 FISH CARE AND LABORATORY CONDITIONS

For behavioural experiments, the breeding male sticklebacks were put straight into the one metre long testing tanks to settle down and commence nest construction. The females were housed in groups of about 15 to 20 fish in similar sized tanks. The tank floors had a layer of gravel and some weed cover. All fish were fed daily with Tubifex worms and occasionally with Daphnia. As a prevention against disease, water in the tanks containing the females was changed every one or two weeks; the test tanks that contained the males were thoroughly cleaned after testing with any one particular male. All fish in the laboratory during the breeding season were kept in a light regime of 16 hours light/8 hours darkness.

The trout used as predators of sticklebacks in the breeding tests were obtained from the west of Scotland Trout farm at Brigde of Weir, Scotland. After trying several systems, they were found to survive best in large fibre tanks of two metres diameter and one metre deep and with provision for circulating water. They were fed daily on trout pellets.

2.4 STATISTICAL ANALYSIS

All the statistics in this study were carried out after consultation with the Statistics Department of the Glasgow University. Parametric tests were carried out where appropriate, otherwise non-parametric tests were used. The Minitab package was used for all the parametric tests.

CHAPTER 3

AGE STRUCTURE OF THREE SCOTTISH STICKLEBACK POPULATIONS

3.1 INTRODUCTION

A feature of the growth pattern of the three spined sticklebacks (these fish may be referred to as sticklebacks) is the great variation in their life span both within and between populations. One possible source of variability might be that there are two subspecies of three spined sticklebacks (Wootton, 1976). *Trachurus* is the larger and fully plated form which overwinters in the sea and migrates into fresh water to breed (anadromous). *Leiurus* is the partially plated form which usually remains in fresh water throughout the year. Bertin in 1925 (cited by Wootton 1976) studying many sites in France and Netherlands concluded they had a life span of just over a year. It has been suggested that the *leiurus* form could live for up to five years in well maintained aquaria (Bock 1928), but what happens in the protected world of the aquaria might bear little resemblance to what happens in nature. Craig-Bennett (1931) found that the fish in his *leiurus* population in Cambridge did not breed until two years old and had a maximum life span of at least three years. Most of the *leiurus* sticklebacks in River Birket in Cheshire bred in their second summer of life when about a year old and lived for up to three or four years (Jones and Hynes 1950). Greenbank and Nelson (1959), working on *leiura* in two lakes in Alaska, gave similar results. Their fish bred when one or two years old and had a life span of about two and a quarter years. The *leiurus* sticklebacks in two streams in Southern England lived for just over a year (Mann 1971). Similar results were obtained by Allen and Wootton (1982) who worked on three spined sticklebacks in Llyn Frongoch and Giles (1981) while investigating some Scottish populations. Most of the *leiurus* sticklebacks in these studies bred in their second summer of life

when just a year and few survived for a second year. Considerable variability thus exists and most authors agree that while some of these differences may be inherited, various aspects of the environment in which the fish lives are likely to be important.

The objective of the present study was to investigate age structure of sticklebacks in three Scottish populations for comparison with existing literature. It will also be related to other aspects of stickleback biology such as gonadal development (chapter 5) and food intake (chapter 4).

3.1.1 Techniques for ageing fish

Several techniques have been developed for ageing fish and most of these methods have been extensively used by different workers. There are drawbacks to each of these methods but usually one is superior for a given species. One or more of the following methods have been used:

(1) Annuli method

This involves counting and interpretation of characteristic marks which appear at regular intervals during the growth of bony structures in temperate zone fishes (Mann 1971; Kimura et al 1979). Scales, otoliths and opercular bones are commonly used. Some of these marks which are considered to be formed once a year appear as the fish alternate between periods of faster and slower growth or no growth at all and the number of marks reflects the fishes' age. The rationale in their use is that:

- (a) Growth is continuous but not constant.
- (b) Growth is slowed down by such factors as low food supply, breeding and low temperature.
- (c) Periods of fast and slow growth produce bone which looks different.

One then needs to interpret exactly what set of rings results from the different conditions. Jones and Hynes (cited above) as well as Greenbank and Nelson (1959) concluded that in different species, the formation of the opaque and transparent rings may occur during different seasons and that the season of formation was constant for a given species.

(2) Size frequency distribution method

This is derived from frequent sampling of a large number of fish species which reproduces seasonally (so that recruitment is pulsed) and individual lengths of a large number of the fish are measured. The existence of peaks in the length frequency histogram indicates the existence of different age classes. This method requires a unimodal size distribution of all fish of the same age. It is easy to use if there are no large size overlap in individuals of adjacent age groups. However it is less suitable for older fish because as the fish age, the size modes become progressively less pronounced. This technique has been used as the sole criteria for ageing sticklebacks (Mullem and Vlugt 1964).

(3) Marking and Tagging

This method has mostly been used to validate other methods of ageing fish (Le Cren 1947). Fish can be marked when fry or at a known age and then released. When recaptured, the known age should for example correspond with the number of rings on their skeletal structures. There is the possibility however that the marked fish grow more slowly than the unmarked ones. The great advantage of tags is that they can be serially numbered thus enabling individual fish to be identified but most tagging methods cannot be used on small fish like sticklebacks.

(4) Rearing fish in captivity

Fish can be reared in captivity and the number of years the fish has been held is compared to the number of supposed annuli laid down during this period. One criticism of this method is offered. The environment in which the fish is held, is artificial and may bear little comparison to the natural habitat of the fish. Marks laid down by a natural stock of the same species may therefore differ both in number and appearance.

3.1.2 Techniques applied to sticklebacks

The otolith method formulated by Jones and Hynes (1950) for sticklebacks was used to age fish in this study irrespective of the difficulty in dissecting out the tiny sagitta and in interpretation of the rings. The size frequency distribution was used to validate the otolith results.

There has been controversy in the past on which methods are most reliable for ageing sticklebacks. Bertin (1925) based his assessment of stickleback age structure on length frequency distribution and stated that otoliths were not always useful for age determination except when combined with length frequency distribution. Jones and Hynes (cited above) in their study of age and growth of G. aculeatus however found the otoliths the only satisfactory method and found that estimating age using only size distribution could be misleading because of the overlapping of different age groups. Based on this, they showed how Bertin (cited above), who used only length frequency, was misled into the belief that his species lived for only two summers. Mullem and Vlugt (1964) still used length measurements as the sole criterion for determining the age of the anadromous sticklebacks (trachurus). They quoted Munzing (1959) who is of the view that it is highly unreliable to distinguish and count the rings in the otoliths of the trachurus form of sticklebacks.

False annuli in otoliths have been a source of inaccuracy in age determination of fish. Even Jones and Hynes (cited above), who considered the otolith satisfactory, found there were instances (2%) when the otolith was completely uniform with no annulations at all. Giles (1981) and Allen and Wootton (1982) used the method formulated by Jones and Hynes (cited above) and on the basis of indirect evidence found the otolith and length frequency broadly reliable for determining the age of the three spined sticklebacks. Mann (1971) concluded that although there is relatively little substantial evidence (such as observation of otolith in fish of known age) of the soundness of age determination from this method, the sum of indirect evidence (other techniques such as length frequency) leaves

no doubt that in general their use is justified.

Some direct evidence has been given recently that questions the reliability of the use of otoliths to age sticklebacks. Three spined sticklebacks raised under identical conditions in the laboratory were found to have quite different ring formations. This casts some doubt on the value of otoliths for ageing sticklebacks although it might be that some critical factor of natural environment was omitted from the laboratory conditions.

Blouw and Hagen (1981) found the otolith completely unreliable for ageing the four spined sticklebacks (Apeltes quadracus). They found that 20% of both otoliths from an individual fish were unreadable and a similar proportion just had one of the otoliths unreadable. In 50 percent of the cases the two otoliths from the same individual fish differed in their annulations. They also reared some fish under identical conditions in the laboratory and found great variability in the otoliths even between otoliths of an individual fish, among individuals of the same clutch and among clutches of the same age. FitzGerald (pers comm) was also not able to age sticklebacks from their otoliths and attributes this to the highly variable nature of the estuarine environment that these sticklebacks inhabit which resulted in many false annuli.

In the present study, the fish were aged by examining their otoliths, which was validated by looking at their length frequency distribution. The populations studied were exposed to regular seasonal changes but not to the sudden and unpredictable changes experienced by FitzGerald's populations. The otoliths showed definite

annulations in majority of the cases. There were some difficult cases (which are discussed fully later) and in some instances, the body weight of the fish, the gonad weight and its histological state, together with the time the fish was captured were all taken into consideration in ageing the fish. All this information allowed the fish to be aged in the majority of cases.

3.2 MATERIALS AND METHODS

3.2.1 Sampling regime

The three populations were sampled unsystematically at approximately six weekly intervals between October 1982 and August 1983. During each visit 20 fish were brought back to the laboratory; measurement of their standard length (tip of snout to the base of the caudal peduncle) was taken using vernier calipers to the nearest 0.1mm. Fish of different lengths were grouped together in categories as shown below to make analysis easier.

Length Category	Fish length (cm)
1	1.5 - 2.0
2	2.1 - 2.5
3	2.6 - 3.0
4	3.1 - 3.5
5	3.6 - 4.0
6	4.1 - 4.5
7	4.6 - 5.0
8	5.1 - 5.5
9	5.6 - 6.0

10	6.1 - 6.5
11	6.6 - 7.0

Their otoliths were then extracted for ageing purposes. From September 1983 to August 1984, in order to study the age structure of the populations in more detail, monthly samples were taken. During this later period, about 100 - 200 fish were collected and measurement of their standard length taken. Twenty fish, a few from each size category, were then taken back to the laboratory for otolith and other analyses and the rest of the fish were returned to the river. The analysis which follows refers mainly to the 1983-84 samples but the results were compared to the earlier samples.

3.2.2 Preparation and interpretation of otoliths

To extract the otoliths, the top of the cranium was removed with a sharp scapel, the cut extending from the back of the skull to the back of the eye. Any remaining part of the medulla oblongata was removed to expose the sagittae which were then picked up with fine forceps. The two sagittae were washed in distilled water, dehydrated in absolute alcohol for 30 minutes and then transferred to pure creosote oil for ten to fifteen minutes. Once cleared, they were mounted on a glass slide using DPX and examined against a black background with reflected light at x40 magnification.

3.2.3 Assigning fish to age classes

All fish brought back to the laboratory were assigned to age classes for the purposes of relating age to length, weight and food intake as

will be seen in later chapters. In doing this, April of the year the fish was bred was taken as the average date of birth for fish since breeding usually commenced around this time. Fish were assigned age 0 from this time until the following April when they became one or two years accordingly.

3.2.4 Breeding in early and late hatched fish

It became apparent at an early stage in the preliminary sampling programme that sticklebacks were being recruited into all the three populations as late as August and September and that by the following spring, some fish were still very small. In order to find out whether these small fish reached sexual maturity in the next breeding season after they were bred, groups of fish of the smaller size categories (2.1 to 3.5 cm) were kept in large holding tanks from April (beginning of the breeding season) to August (end of the breeding season) and their breeding condition noted. The tanks were kept outside at natural day length and temperature and fish were given an abundant supply of tubifex worms and Daphnia. Also from the beginning of the breeding season, early and late hatched broods were kept in separate holding tanks until the beginning of the breeding season in the subsequent year. This was to ascertain that fish came into breeding condition in their first year and to follow the reproductive history of fish hatched at different times of the year.

3.2.5 Data analysis

The age structure of the three populations was analysed by calculating the percentage of fish in each sample which fell in the

various otolith categories (see next section) and by plotting monthly length frequency histograms.

3.3 RESULTS

The results described in this section are based on the regular monthly samples taken from September 1983 to August 1984. The results from the pilot studies conducted from October 1983 to August 1984 agree with these.

3.3.1 Growth of otoliths

The pattern of ring formation was similar for the three populations of this study. The exact time the rings appeared however varied slightly between populations as well as in individual fish. The centre (C) and an opaque (O) ring first appears in their first summer of life. A transparent (T) ring is then added and this alternates with an opaque ring as the fish ages. Therefore the categories of otoliths recognized were as follows:

- (a) OO (Plate 3.1), typical of fish in their first summer.
- (b) OOT (Plate 3.2), typical of fish in their first autumn.
- (c) COTO (Plate 3.3), typical of fish in their first spring/second summer.
- (d) COTOT (Plate 3.4), typical of fish in their second autumn.
- (e) COTOTO (Plate 3.5), typical of fish in their third ^{second} spring/^{third} summer.

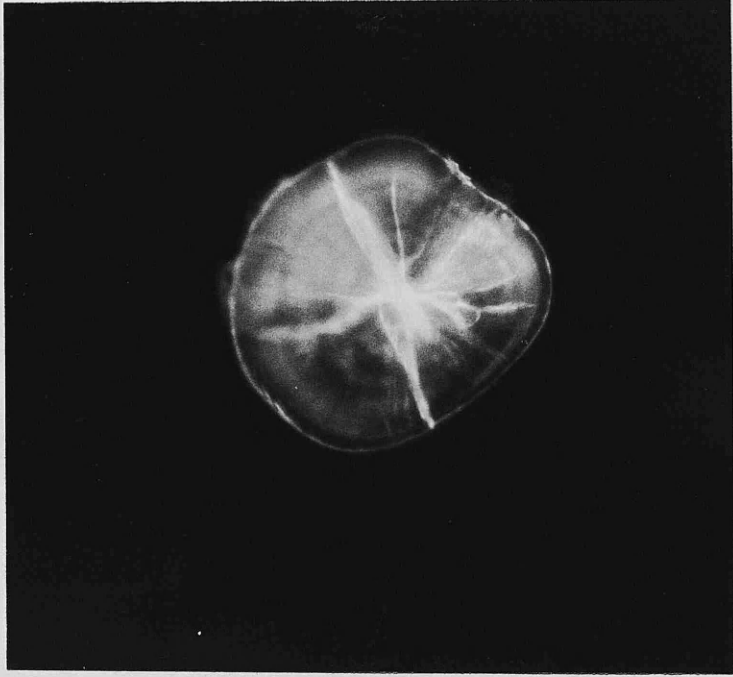


Plate 3.1 Otolith assigned to category (CO), with a centre and one opaque ring.

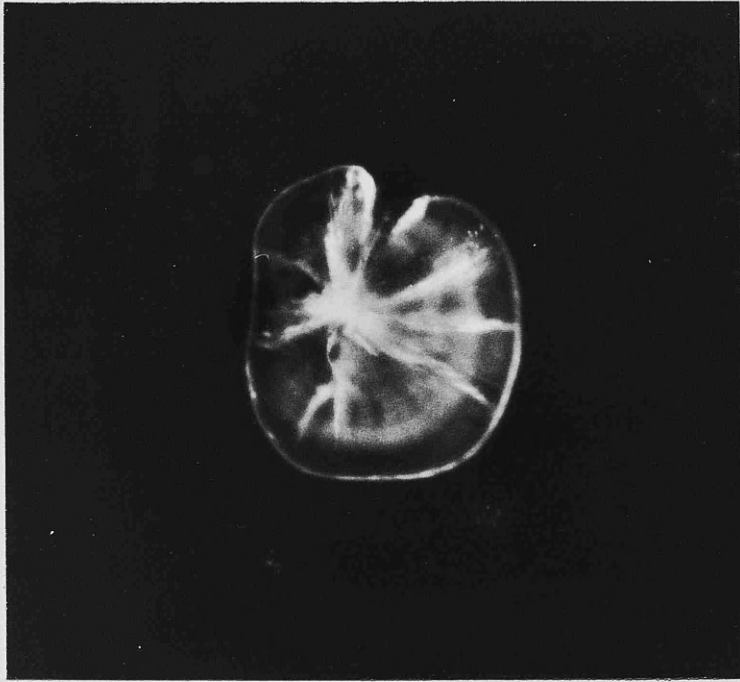


Plate 3.2 Otolith assigned to category (COT), with a centre, one opaque ring and one transparent ring .



Plate 3.3 Otolith assigned to category (COTO), with a centre, two opaque rings and one transparent ring.

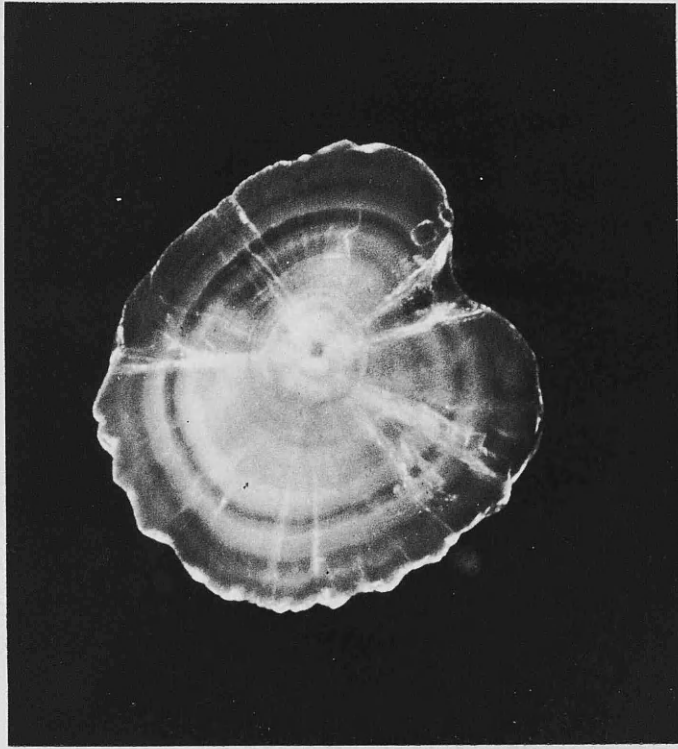


Plate 3.4 Otolith assigned to category (COTOT), with a centre, two opaque rings and two transparent rings.

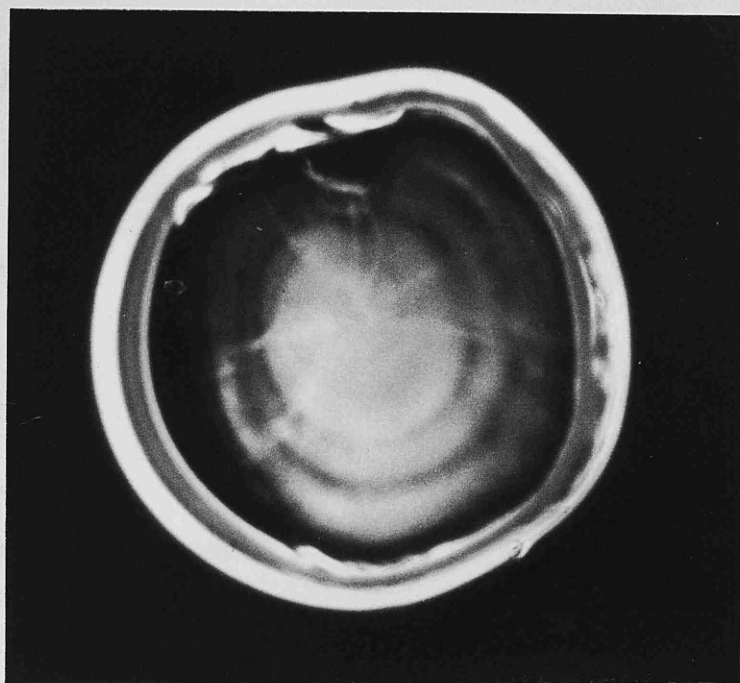


Plate 3.5 Otolith assigned to category (COTOTO), with a centre,
three opaque rings and two transparent rings.

3.3.2 Typical difficulties encountered in interpreting otoliths

(1) Some otoliths had no demarcations at all, being uniformly opaque. This occurred mainly in the large sized fish (6cm and above). It accounted for 1.2% of all the Kelvin and Aurs Burn fish and 0.9% of the Luggie fish that could not be aged on the basis of their otoliths but their size suggests they are at least two years old (Plate 3.6).

(2) A few false annulations (small irregular rings) in otoliths was observed for Aurs Burn only (0.9%)

(3) The left and right otoliths were different in some instances. This was observed in Rivers Kelvin (0.9%) and Aurs Burn (0.3%) only and was usually seen when one otolith had many false annulations or when a ring has been added in one and not in the other. One otolith usually showed the most likely age taking into account the length of the fish and the time of capture

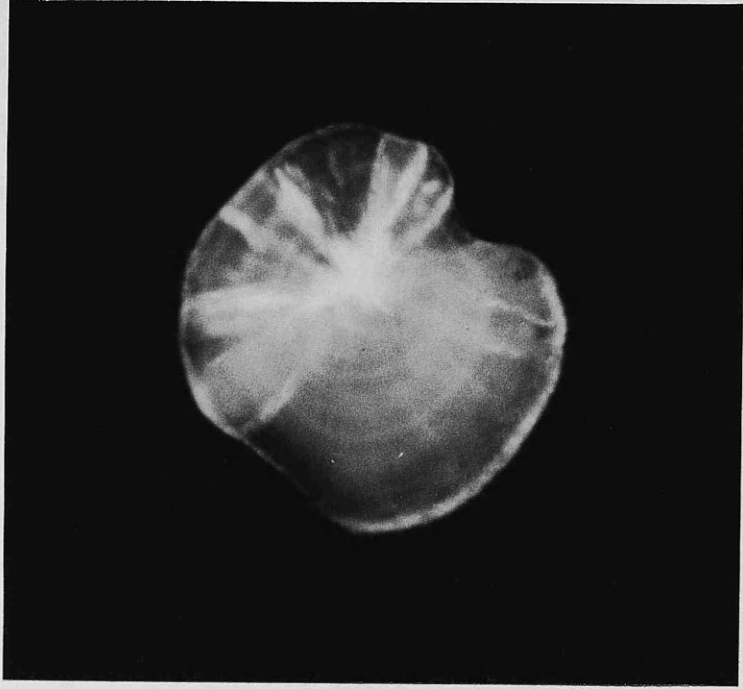


Plate 3.6 An example of an otolith with no demarcations and which is uniformly opaque.

(4) In some cases, a relatively big fish had fewer rings compared to a much smaller fish. The otolith alone would suggest young of the year fish while the length and weight of the fish indicates an adult. If this occurred during or immediately after the breeding season i.e September, a histological examination of their gonads sometimes showed collapsed tissues for the females (Plate 5.4, chapter 5) and empty tubules for the males (Plate 5.7, chapter 5). This suggested that these fish had bred and therefore were adults.

(5) In very few instances, the reverse was the case. The otolith suggested an adult fish while the other parameters suggested the fish was a young of the season. These cases occurred in the Kelvin (0.9%); Luggie (0.4%) and Aurs Burn (1.2%).

Inspite of these very few instances that presented difficulties with otolith analysis of some individual fish, a clear picture of the age structure of the populations studied as a whole was obtained. Age and length agreed well, so that 97% of the River Kelvin population, 96.7% of the Aurs Burn and 98.7% of the Luggie could be aged with a high degree of confidence. The remaining fish were omitted from the analysis.

3.3.3 Pattern of ring formation

River Kelvin

Table 3.1 shows the frequency distribution of otolith types and the mean and range of the standard lengths of sticklebacks in each category. A centre and opaque ring is laid down soon after hatching. By July 1984, a transparent ring had already appeared in some young fish. All CO's and COT's in July and August 1984 are young of the year. All adults at this time were COTO and about a year old except one fish that had COT but was clearly an adult considering its length, weight and gonad state. By October 1983 all young of the year caught were COT's but even by April 1984, a small number of fish still had a centre and an opaque ring (CO) even though all other parameters suggest that they belonged to the 1983 year class. A second opaque ring was first noticed in the 1983 year class in March 1984 when the fish was approaching a year old; by August all adults had this second outer opaque ring. The second transparent ring started being formed in the autumn when the fish were over a year old; very few fish survived to this age. A fish was however caught in June 1984 which had added a third opaque ring suggesting that it was two years old. Three similar sized fish were also caught but had no annulations and so could not be aged on the basis of their otoliths.

Table 3.1 Frequency distribution of otolith types and mean and range of standard length of the fish in each category for River Kelvin.

	Percentage distribution of otolith category				
	CO	COT	COTO	COTOT	COTOTO
September 1983	24	38.4	9.6	28	
October 1983	-	72	4	24	-
November 1983	-	84.8	-	15.1	-
December 1983	-	89.6	-	11.2	-
January 1984	-	95	-	5	-
February 1984	5	95	-	-	-
March 1984	-	95	5	-	-
April 1984	5	85	5	5	-
May 1984	-	91.2	9.6	-	-
June 1984	25	55	15	-	5
July 1984	51.6	21.5	25.8	-	-
August 1984	30	65	5	-	-
No. of fish in each class	34	218	43	21	1
Mean standard Length (cm)	3.1	4.0	4.4	5.2	6.1
Range (cm)	2.2-5.1	3.4-4.6	3.6-4.4	5.0-6.0	-

RIVER LUGGIE

Table 3.2 is the frequency distribution of otolith types and the mean and range of the standard lengths of sticklebacks in each category. All young fish in their first summer of life (in July and August 1984) had a centre and opaque ring. No young fish had a transparent ring at this time, all COT's and COTO's being one year adults. A first transparent ring was observed in young fish in September 1983 and remained the outermost ring of all the 1983 year class until April 1984. A second outer opaque ring was first observed in May 1984 when the fish was about a year old. The second transparent ring was first noticed in fish caught in October 1983 when they were just over a year old. Fish of this otolith category was however not caught after January 1984, giving the population a maximum observed life span of about eighteen months. The biggest fish caught in this population was 5.9 cm in October 1983 but had no demarcations in its otoliths. It is likely to be just over a year old, belonging to the 1982 year class.

Table 3.2. Frequency distribution of otolith types and mean and range of standard length of the fish in each category for River Luggie.

	Percentage distribution of otolith category				
	CO	COT	COTO	COTOT	COTOTO
September 1983	-	20	80	-	-
October 1983	-	?	?	?	?
November 1983	15	85	-	-	-
December 1983	-	84.2	-	15.8	-
January 1984	31.6	63.2	-	5.2	-
February 1984	5	95	-	-	-
March 1984	-	100	-	-	-
April 1984	-	100	-	-	-
May 1984	-	68.4	31.6	-	-
July 1984	85	5	10	-	-
August 1984	60	40	-	-	-
No.of fish in each class	63	147	9	6	-
Mean standard length (cm)	3.2	3.6	4.1	5.5	-
Range (cm)	2.9-4.0	2.5-4.4	3.8-4.6	5.5-5.6	-

AURS BURN

Table 3.3 shows the frequency distribution of otolith types and the mean and range of the standard lengths of sticklebacks in each category. As in the River Luggie, all young of the year fish had a centre and opaque ring by August. The first transparent ring was first noted in young fish caught in September at which time all the adults caught were either COTO or COTOT. The second opaque ring in the 1983 year class was first observed in May 1984. The one fish with COTO in January belonged to the 1982 surviving year class (on the basis of size) which had not added an outersecond transparent ring. All CO's from January 1984 right through to May 1984 belonged to the 1983 year class, as well as all COT's. The second transparent ring was first observed in September 1983 and was seen in a fish in February 1984 when the fish was about nineteen months old. The biggest fish caught in this population was 6.5 cm in November but the otoliths had no demarcations. Considering it's length and weight, the fish was probably over two years old. Another large sized fish of 6 cm in July also had otoliths with no demarcations.

Table 3.3 Frequency distribution of otolith types and mean and range of standard length of the fish in each category for Aurs Burn.

	Percentage distribution of otolith category				
	CO	COT	COTO	COTOT	COTOTO
September 1983	73.1	15.4	7.7	3.8	-
October 1983	-	72	-	28	-
November 1983	-	100	-	-	-
December 1983	5.3	89.4	-	-	-
January 1984	50	44.4	5.6	-	-
February 1984	25	70	-	-	-
March 1984	25	75	-	-	-
April 1984	5.9	94.1	-	-	-
May 1984	10	85	5	-	-
July 1984	90	10	-	-	-
August 1984	65	20	15	-	-
No of fish in each class(cm)	73	173	34	10	-
Mean standard length (cm)	3.3	4.0	4.7	5.6	-
Range (cm)	2.7-4.5	3.3-4.9	4.7-5.1	5.1-5.9	-

3.3.4 Length frequency Distributions

Figs. 3.1 to 3.3 show the length frequency distributions for the three study populations. For most of the year in each population the distribution is unimodal, dominated by fish bred the previous breeding season. Young of the year fish appear in June in the River Kelvin and the distribution becomes bimodal, consisting of the young of the year and the breeding adults. This bimodal distribution could be seen in the Aurs Burn in August but did not quite show up in the River Luggie during this period. The length frequency histogram becomes unimodal again by January as the few surviving adults left as tail die out by the end of winter in all 3 populations.

By April the fish had reached a modal size of 4 to 4.5 cm in the Kelvin and Aur Burn but not in the Luggie until May but range still remained large due to the presence of late hatched fish. Small fish of about 2 cm (weighing .1615 gm) continued to appear in the populations as late as September when some young of the year were already 4.2 cm (weighing .7654 gm.). This suggests a more protracted breeding season than is common for most stickleback populations and is one reason why within the same broad age category of young fish length and weight can be very different.

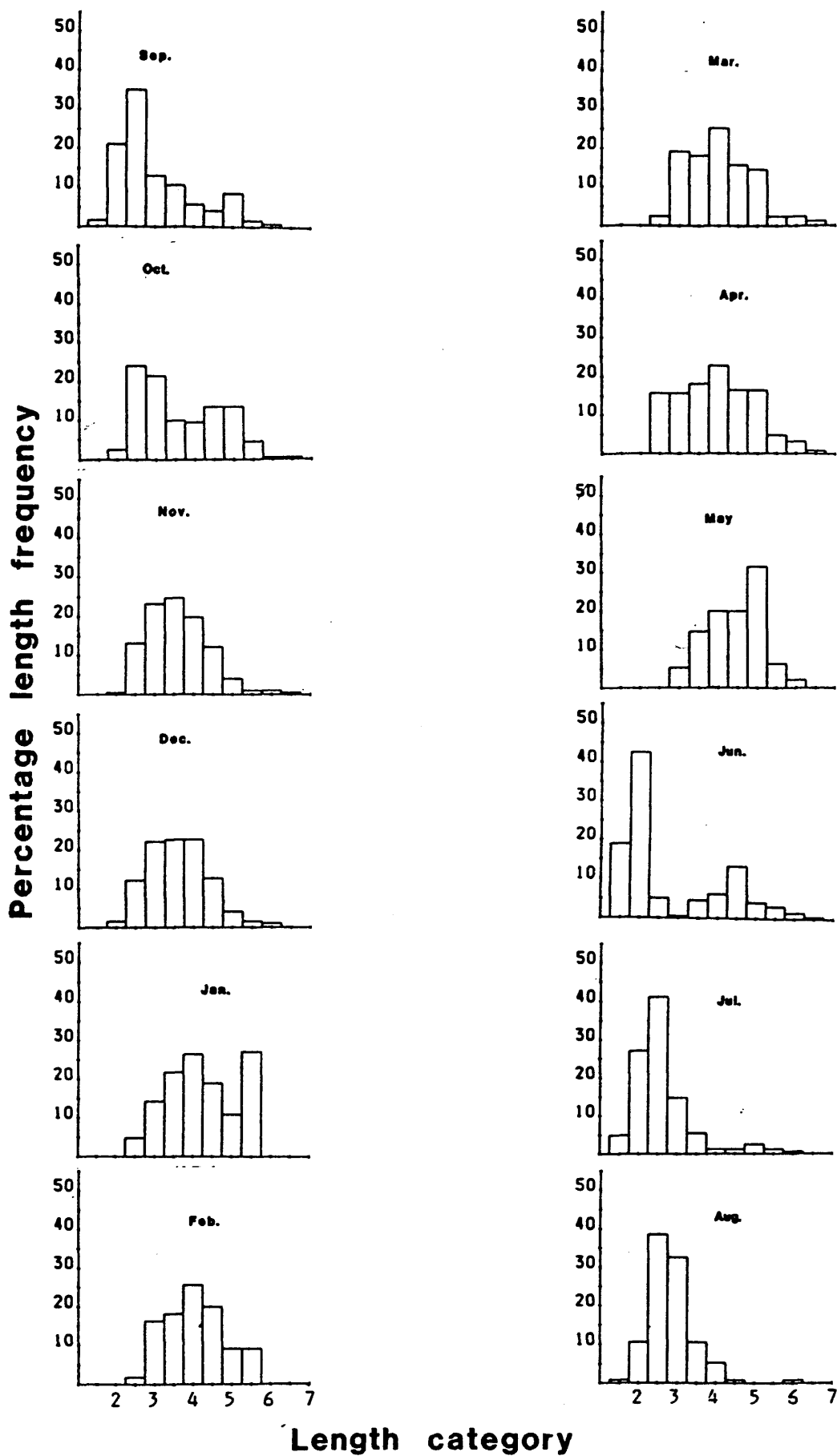


Figure 3.1 Length frequency distribution for the months of January to December for fish from the River Kelvin.

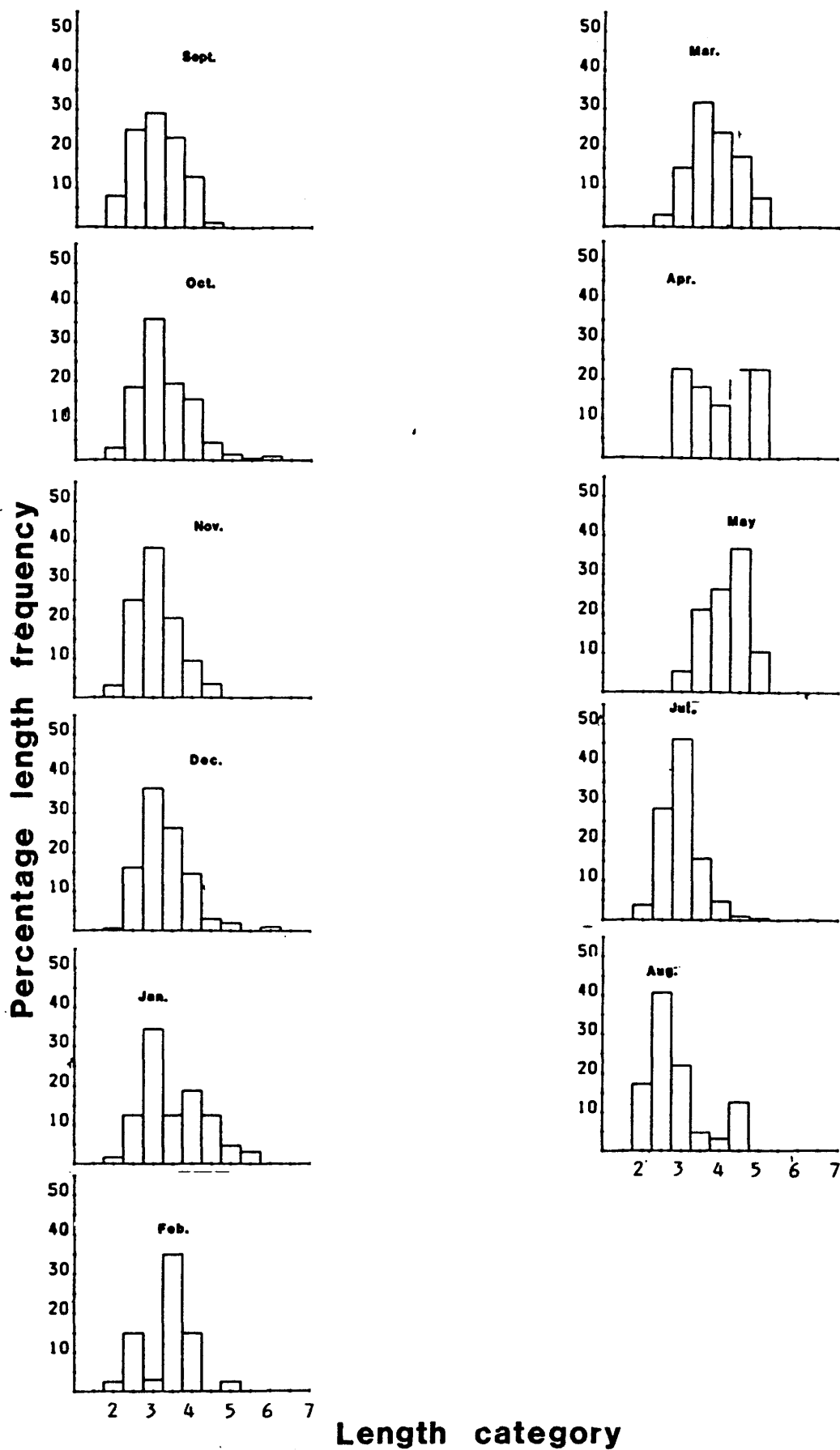


Figure 3.2 Length frequency distribution for the months of January to December for fish from the River Luggie.

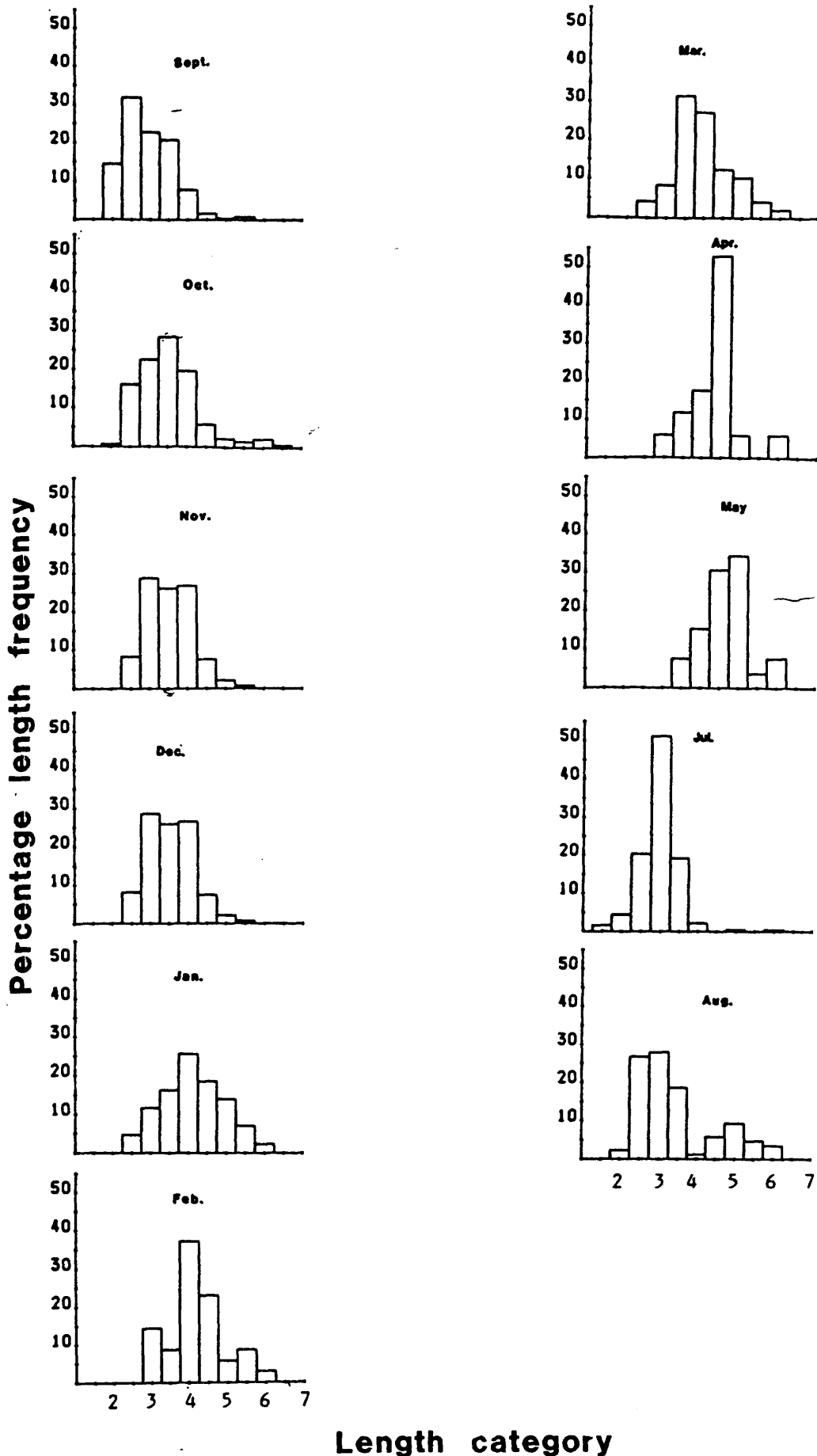


Figure 3.3 Length frequency distribution for the months of January to December for fish from the Aurs Burn.

3.3.5 Population age structure

The two methods of ageing fish used in this chapter gave similar results. Though breeding started in late April, recruitment of young fish (from 1.5 cm and above) was observed by June in the River Kelvin (Luggie and Aurs Burn were not sampled in June) and in July, this was observed for the River Luggie and the Aurs Burn.

3.3.6 Breeding in early and late hatched male sticklebacks

For the late hatched fish kept in large holding tanks at the beginning of the breeding season, those around 2.0 cm in spring failed to breed while the majority of fish that were above 3 cm came into breeding condition as the season progressed. In the case of the early and late broods kept in holding tanks at the end of the breeding season, all early broods came into breeding condition at the beginning of the season while none of the late broods did.

3.4 DISCUSSION

The length frequency distribution and otolith analysis used to age sticklebacks in this study agreed with each other well. A clear picture of the age structure of the whole population was obtained even though some individual fish proved difficult to age with confidence. Both the otolith and the length frequency distribution show that a great majority of the fish from the three populations have a maximum life span of just over a year. Both the data described in the previous section and field observation indicate that a mass mortality of adult fish occurs immediately after the breeding

season. In the Kelvin and Aurs Burn females however a few survived to two years. This pattern of age structure has been reported by Giles (1981), who carried out a study of the ecology of some Scottish populations including River Luggie and by Allen and Wootton (1982) who worked on the Llyn Frongoch population in North Wales.

Use of otoliths for ageing sticklebacks was first formulated by Jones and Hynes (1950) but the pattern of ring formation obtained from this study was different, with opaque and transparent rings occurring during different seasons. It was however generally similar to the pattern showed by the Llyn Frongoch population, although the timing of appearance of the opaque and transparent rings were slightly different. All fish in Llyn Frongoch had a first transparent ring by the October after they were bred but in the present study, some fish were still without it by the January following breeding.

In the present study there was much variation among individual fish as to the timing of appearance of rings; for example, almost one third of the young of the previous season fish were still without the first transparent ring in January in the River Luggie and more so for the Aurs Burn. In Llyn Frongoch all fish had obtained their first transparent ring by October. It was observed in the present study that formation of rings varied slightly between individual fish as well as between populations, in contrast to suggestions by both Jones and Hynes (1950) and Greenbank and Nelson (1959) that the season of formation of rings was constant for a given species. The first transparent ring was for instance formed in the Kelvin young by July but did not appear in the other two populations until September. Among fish bred in the same season, a smaller fish might have an

outer transparent ring (COT in November) while a bigger fish might still only have a centre and an opaque ring. Broadly speaking, bigger fish have more rings, but there is a clear size overlap between otolith types so that size alone is not adequate.

The sticklebacks of the three study populations commence breeding about late April and continue until August. The earlier broods of fish grow fast in their first months of life and are able to reach maturity and breed in their second summer when they are about a year old. In contrast, many fish from the later broods are still not mature at the beginning of the breeding season. Some are able to reach maturity and breed later on in the season while others do not breed at all in their first year. Although this later group of fish could potentially survive to breed for the first time in the next season when they are about 20 months old, the data from the present study suggests that this is unlikely for females and even more so for males.

Giles (1981) and Allen (1980) both noted a similar group of fish that did not breed in the first breeding season (after hatching) in their studies and Allen suggested that those fish that survived a second winter were those which had failed to breed at age one either due to their small size, parasite load or food shortage. Wootton (1976) reports that "if the critical minimum size for maturity is not reached when the fish are one year old, breeding will not take place until the next breeding season when they are two years". Very few fish survived to be two years old in the present study and were females in all cases. So effectively, late hatched males of the three populations which fail to reach breeding size in the subsequent

breeding season do not breed at all.

The fish hatched early in the season in the present study populations could be up to 4.3 cm and weighing just under a gram by September when they are about 4 months old. The Karluk lake leiurus form of sticklebacks studied by Greenbank and Nelson (cited above) had a total length of 4 cm at the end of their first summer and by 15 months averaged about 6cm. Van Mullem (1967) under controlled conditions observed his sticklebacks could reach 4.5 cm in as little as 4 months by which time they were sexually mature. The fish bred early in the season in this study had quite similar growth rates in their first months of life. The growth rate of the Llyn Frongoch population of sticklebacks studied by Allen and Wootton (cited above) is amongst the slowest recorded. By the time they were 15 months old, they attained a total length of approximately 4.1 cm and this was attributed to food shortage.

Some fish in the present study were about 5 cm on average when about 10 months old and weighing over 2 grams while at the same time there were some other fish bred at the same season but which were only 2.7 cm in length and weighing just over 0.2 grams. The only reason found for this small size was the time the fish were bred which was towards the end of the season when food supply was not as rich as in the beginning of the breeding season. The gonadal development of the fish was looked at in chapter five in attempts to monitor their rate of maturing and relating this to size, among other things.

CHAPTER 4

FEEDING INTENSITY AND DIET COMPOSITION

4.1 INTRODUCTION

As discussed in the Introduction and demonstrated for the present study populations in chapter 5, there is considerable variability in growth rate and life span of sticklebacks. Food supply has been shown to be one of the major factors which affect these traits in many fish species. Constantz (1974) compared body size and fecundity (by counting and weighing the eggs in a mature female) in top minnows in the head of a spring where food availability was low and in a downstream portion which offered more food. On average, downstream fishes were 19% longer and 440% more fecund. In the Japanese medaka (Oryzias latipes), fish fed little food in the laboratory under controlled temperature conditions sacrificed growth rather than reproduction, while fish fed high levels of food put more into growth and survival (Hirshfield 1980).

Experimentally imposed differences in food availability have also been shown to cause changes in growth rate, fecundity and age or size at maturity in trout species (Alm 1949), in rainbow trout (Salmo gairdneri), (Scott 1962) and in the three-spined stickleback (Wootton 1973). As food supply was increased experimentally, fish grew faster, and age at maturity decreased.

Female sticklebacks are particularly sensitive to food supply especially during the breeding season, when their main role is the production of eggs and spawning them in the nest of the male. Producing eggs is costly and has been shown to be positively correlated with ration (Wootton and Evans 1976). As food supply becomes low, female sticklebacks channel a greater proportion of the

energy content of their food into egg production than they would at higher food levels (Wootton 1977).

Gamete production for the male stickleback is less energetically expensive, but heavy investment in parental care means both energy expenditure and a reduction of the time available for feeding. Stanley and Wootton (1986) observed that the proportion of males that acquire a territory and construct nests successfully increases with increased food supply. Males also lost weight over the breeding cycle except when they were on a very high diet. So there are heavy energetic costs of breeding for males, although the costs are different to those incurred by females. Food is therefore important in growth and reproduction of both male and female sticklebacks; reproductive output increases at high food levels. Food intake and its relationship to growth and development was therefore looked at in this study and discussed in the next two chapters.

The literature on the food of sticklebacks is quite extensive. Various attempts have been made to assess competition for food between sticklebacks and other species of fish especially those of commercial importance (for example Maitland 1965; Rogers 1968). Others have been mainly concerned with the quantitative estimation of the rate of food consumption of sticklebacks and the composition of their diet (Hynes 1950; Manzer 1976). Thus many workers have been interested in the season of maximum feeding activity as well as the total daily or annual weight of food consumed by sticklebacks.

Hynes (1950) was the first to do a detailed study of the food of the fresh water sticklebacks (Gasterosteus) and (Pungitius) and showed

that their diet consisted chiefly of crustaceans and insects and that the food taken changes slightly with season and with fish size. Small fish were not able to consume the relatively large organisms that the bigger fish fed mostly on. He also observed low food levels in winter and attributed this to low food availability rather than to low metabolic rates resulting from the low temperatures of winter.

Manzer (1976) studied seasonal and diel changes in feeding habits of sticklebacks and the influence of such factors as size and sexual maturity. He found that the mean weight of the stomach contents was high in spring and early summer and that mature males had fuller stomachs than did mature females. This author also concluded that seasonal changes in diet were caused by changes in the density or availability of the prey rather than by the changes in the preferences of the sticklebacks. Greenbank and Nelson (1959) studying food of sticklebacks in two lakes in Alaska observed differences in the food selected by fish of various size groups; bigger sized fish were eating the bigger prey items.

Abdel-Malek (1962) provides the only detailed study of the food of young sticklebacks (below 1.8 cm) and found that the nature of their feeding changed with age. There was a dietary progression from copepod nauplii - copepodite stages - copepods and by the time the fish reached 1.5 cm, their diet overlapped with that of adults.

Laboratory studies have also been carried out to investigate the amount of food taken by sticklebacks. Rajalsita (1980) calculated the food consumption of the three-spined sticklebacks at different temperatures by determining the rate of digestion and estimated that

sticklebacks consume a greater percentage of food relative to their body weight at higher temperatures. Cole (1978) showed that sticklebacks maintained a full stomach when given food in excess.

Allen and Wootton (1982) estimated the natural rate of food consumption in the field by using regression equations obtained from laboratory experiments on the relationship between growth, food consumption, body size and faecal production of fish over the range of temperatures that the fish experienced in the wild. The natural rate of food consumption in the field was then predicted by first obtaining field measurements of growth, faecal production and temperature and then by extrapolation from these regression calculations obtained from laboratory studies. They concluded that this lay between 3390mg and 5600mg per fish per year. They also found a decrease in feeding intensity during the winter months and an increase in the summer when the fish are breeding.

The work described in this chapter is intended to supplement existing information on feeding biology of the three spine sticklebacks. In particular, its aims are:

- (1) To measure feeding intensity from three populations in Scotland for comparison with existing literature.
- (2) To look at the effect of age, sex and season on these parameters.
- (3) To relate any differences in feeding intensity to size of the fish and to morphological development in the subsequent chapter.
- (4) Also, as a subsidiary aim, to look at diet composition.

Because the primary aim was to relate actual food intake to morphological characters, given the time constraints of this project, food available to the fish was not studied.

4.2 MATERIALS AND METHODS

The stomachs were dissected out of the twenty fish brought back to the laboratory from the three study sites on the sampling occasions (between October 1983 and August 1984) as described in Chapter 2. The fresh weight of each full stomach was taken to the nearest 10^{-4} grams and the stomach was then dissected longitudinally; the food contents were scraped out using forceps and a fine needle. The lining of the epithelium of the stomach tended to come out with the food but care was always taken to separate it out and place it with the empty stomach. The stomach was then reweighed empty and the difference was calculated to obtain the actual weight of the food eaten. The food contents were put in numbered vials and preserved in 70% alcohol for further analyses; this involved identification of the different food types eaten by the fish. To do this, the food contents were put in a petri dish still preserved in the alcohol and were then examined with a binocular microscope, using x40 magnification. All food items in each stomach was then identified as far as possible. In June 1983, no samples were obtained for the River Luggie and Aurs Burn due to unavoidable circumstances.

4.2.1 Stomach Analysis

Methods of stomach content analysis were reviewed by Hynes (1950) and a more critical review was given by Hyslop (1980). Of the five

* The fish used in this study were collected primarily to look at otolith state and gonadal development, in order to clarify age and breeding pattern of the populations, the major aim of this thesis. However a secondary aim was to try and relate these parameters to some measure of food intake. Ideally this would be measured by monitoring the food availability in the three study sites and by looking at faecal production, but this information was not available due to practical constraints of time and transport. What is available is information on the stomach content of the fish. Percentage stomach fullness was calculated as indicated and obviously depends both on the rate of food intake and the rate of stomach emptying. Since Aurs Burn fish appear to be eating chironomidae rather than asellus and gammarus and since the former may be digested more rapidly, percentage stomach fullness may underestimate food intake. Therefore, percentage stomach fullness may not be correlated with food availability. None the less, with this reservation, it was considered desirable to make use of this information.

methods discussed by Hyslop, the gravimetric method was used to determine stomach fullness, while the occurrence method was used for diet composition.

Stomach fullness

This involves determining the weight of the food contents either "wet" or "dry" weight as these have been shown to be highly correlated (Glen and Ward 1968). Different workers have used various modifications of this method. Gibbons and Gee in 1972 expressed the total weight of food contents of fish as a percentage of the weight of the fish. This measure of food content relative to fish size allows for differences in fish size and variations in this have been used in determining the diel rhythm of feeding behaviour of fish (Staples 1975). Kimball and Helm (1971) experimentally determined this for different fish lengths by measuring the amount of water that could be injected into an empty stomach before it bursts. This method of expressing food quantity ^{relative} to the capacity of the stomach gives a measure of stomach fullness which can be used to compare fish of different sizes.

In the present study a stomach fullness index (S.F.I) as used by Allen (1980) was calculated for each fish thus:

$$S.F.I = \frac{\text{Weight of stomach contents}}{\text{Weight of stomach}} \times 100$$

The percentage of fish with empty stomachs was calculated each month for the three populations.

* see opposite

Diet composition

Diet composition was analysed since information on this was available. However, this remains a secondary aim of the study since no data was collected on food availability in the wild for reasons of time. The occurrence method was used, which is perhaps the simplest way of analyzing data from stomach contents (Hyslop 1980). Using this method, the number of stomachs containing at least one individual of each food category is expressed as a percentage of the sum of all stomachs containing food. This could determine the prey species eaten by sticklebacks in the populations and any variations in their relative importance over the year, but gives little indication of the relative amount or bulk of each food category present in the stomach. Hynes (cited above) however concluded that "for any fish with a generalized diet, provided a large number of specimens is examined, any commonly accepted methods of assessing the composition of the diet will give substantially the same results. Those food items important in the diet will always stand out clearly from those that are occasional or rare and so unimportant, and the variations between the different methods will probably not be greater than that between different samples of fish". In the present study, most of the food species in the stomach were identified to class or family. The type of food taken by the different sexes or age classes was not looked at separately. Identification was made possible by the feeding action of G. aculeatus. The food is not chewed even though it may be mouthed several times before swallowing.

4.2.2 Data analysis

5 percent significance level was used in all tests.

Stomach fullness

Seasonal effects: A one way analysis of variance (Anova) was used to test for variations in stomach fullness between months. Comparison between specific months was made using a 2-sample t-test.

Population effects: Any population effect on percentage fullness was looked at using Anova. Pairs of populations were then compared at a time using a two sample t-test.

Sex effects: Any effects of sex on percentage stomach fullness was tested using Anova, while a two sample t-test was used to make month to month comparisons for males and females.

Age effects: Anova was used to test for any effects of age on percentage stomach fullness for all the months of the study. To allow for seasonal changes, the analysis was also carried out just on months with the most variable age structure. These were October to December in the Kelvin; October and August in Aurs Burn and October and December in the Luggie.

Size effects: Anova was used to check for the effects of size on percentage stomach fullness in all months together; then to allow for the possible complications of seasonal effects, the analysis was carried out just on months with similar feeding levels. Table 4.1 shows groups of the months tested for males and females of the three populations. A two sample t-test was used to check for differences in percentage stomach fullness between any two size categories.

Table 4.1 Months with similar percentage stomach fullness, grouped for the purpose of analysis.

Sex	Populations	Months tested
Males	Kelvin	February and March; April; May and June.
Males	Luggie	November to January; April; May July and August
Males	Aurs Burn	April; November to February; May to October.
Females	Kelvin	March and April; May and June; July and August; November and December.
Females	Luggie	November to January; February to May; July; August to October.
Females	Aurs Burn	April and May; November to February; July to October.

Diet Composition

Diet composition was expressed as percentage of occurrence and no statistical analysis was carried out . The food species recorded were Asellus, Gammarus, Cyclops, Chironomid larva, molluscs, algae, plant materials, Ephemeroptera, invertebrate eggs, stickleback eggs and other dipteran larva. Food items such as water beetles, water mites, chironomid pupa and ephemeroptera larva and pupa were exclusive to the River Kelvin population and occurred in just two fish and so were ignored in the analysis. For data analysis, similar food species were combined as follows: 1 = Asellus and Gammarus; 2 = Cyclops; 3 = Chironomidae and other dipteran larva; 4 = Mineral material; 5 = Algae and plant material; 6 = Invertebrate eggs; 7 = Stickleback eggs; 8 = Molluscs; 9 = Ephemeroptera; 10 = all material which could not be identified.

4.3 RESULTS

4.3.1 Stomach fullness

Seasonal effects

There were significant seasonal differences in the percentage fullness of females in all 3 populations and for the Kelvin and Luggie males. Figures 4.1 and 4.2 show seasonal variations of percentage stomach fullness for males and females of the 3 study populations while tables 4.1 to 4.3 show months with significant differences in percentage stomach fullness for the populations.

River Kelvin

All males had full stomachs in August which made use of t-test invalid. The males had high food intake in February, March and August but low intake in December, July and October. All the other months were indistinguishable. For the females, stomach fullness was low in November and December, then there was a gradual increase up to April but only reaching significance by March. A drop occurred in May followed by another gradual increase reaching a peak in August. There was an abrupt drop in October.

Both sexes were therefore eating a lot in early spring but showed a drop in stomach fullness in the early stages of the breeding season; this happened earlier for males. There was an increase in food intake in August for both males and females.

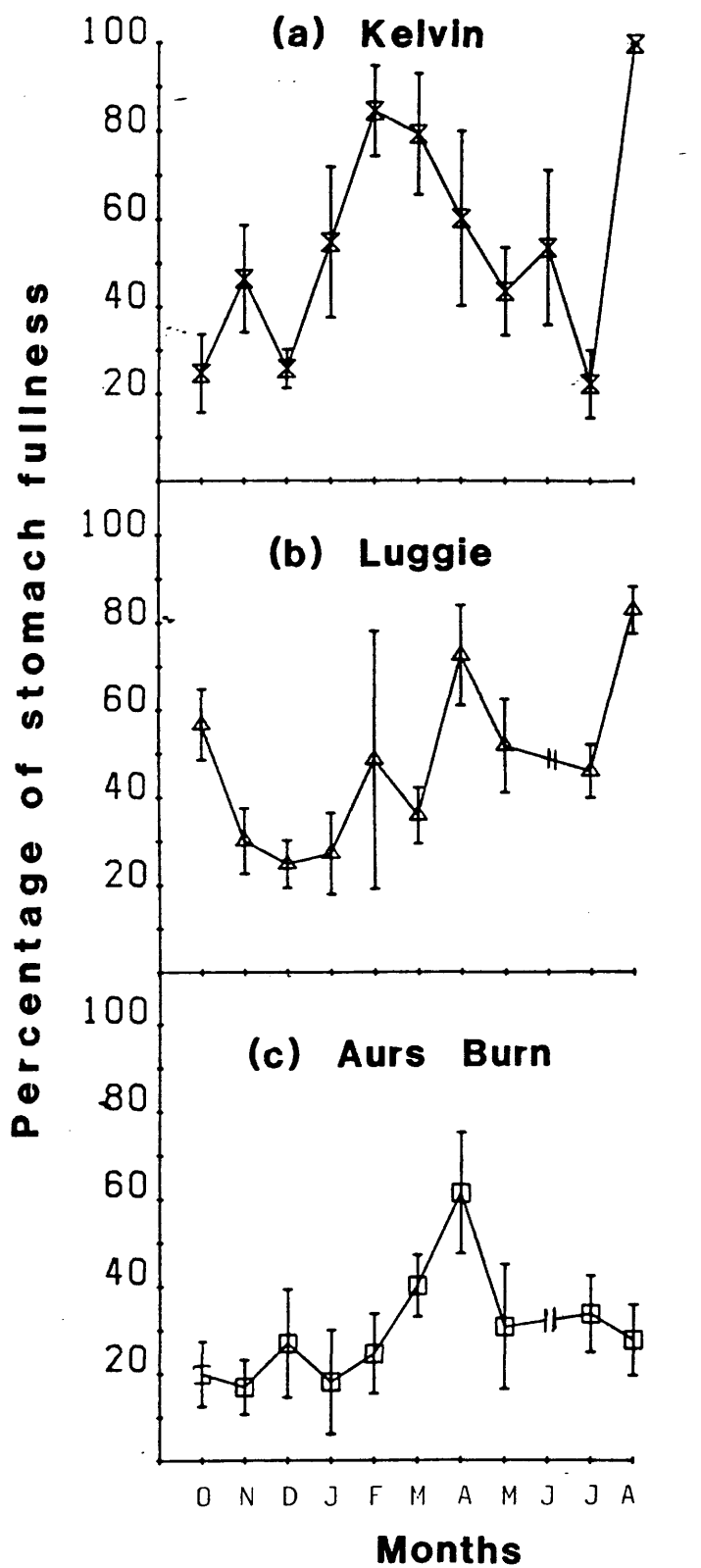


Figure 4.1 Mean and standard error of percentage stomach fullness at different times of the year in male fish from the three study populations

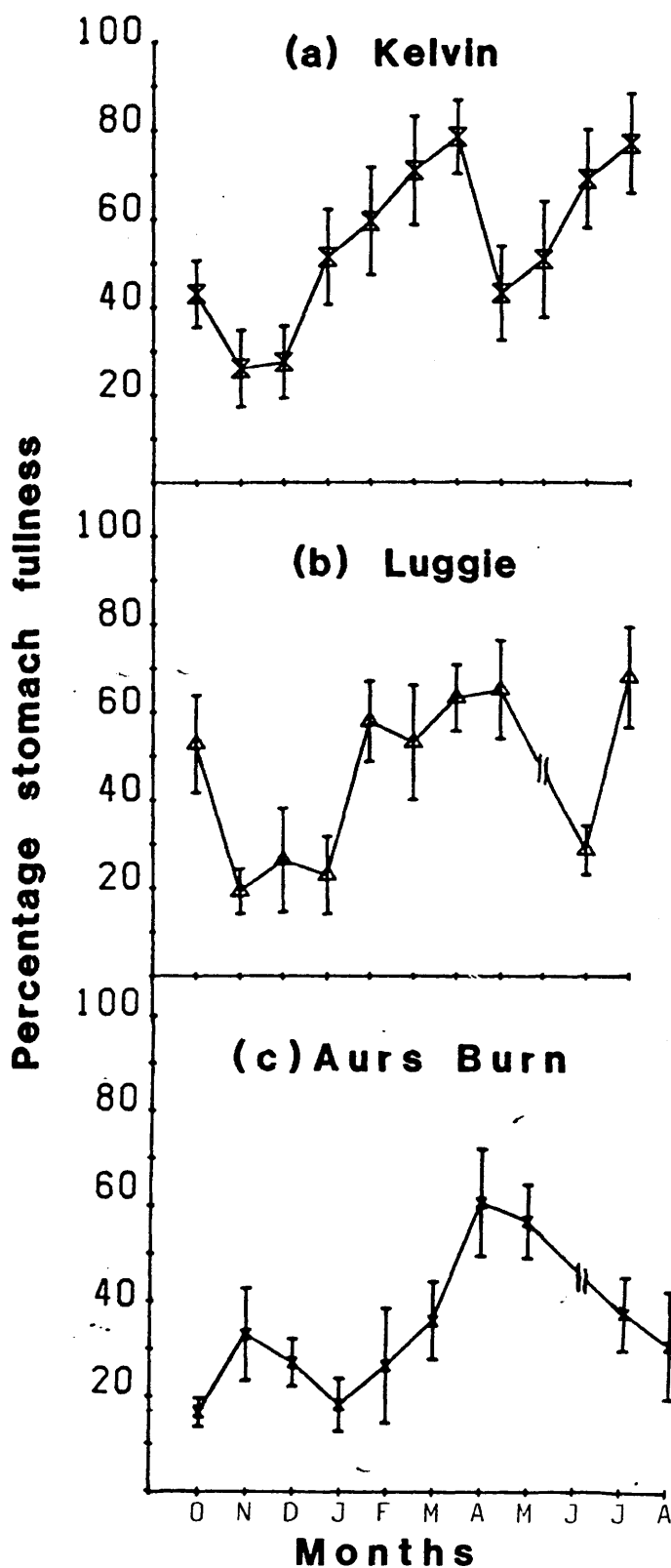


Figure 4.2 Mean and standard error of percentage stomach fullness at different times of the year in female fish from the three study populations.

Table 4.2 Summary of months for which percentage stomach fullness was significantly different: River Kelvin.

Months	Sexes
March and July ($p=.00$)	Males
December and February ($p=.00$)	
December and March ($p=.00$)	
April and May ($p=.01$)	Females
August and October ($p=.02$)	
August and November ($p=.00$)	
August and December ($p=.00$)	
May and August ($p=.04$)	
December and March ($p=.01$)	
December and April ($p=.00$)	
December and July ($p=.00$)	

River Luggie

For males, stomach fullness was low from November to January but rose to a peak in April. A gradual drop occurred in May and July to levels which were still higher than those of November to January. Stomach fullness reached a second peak in August and then dropped in October. Stomach fullness was also low for the females between November to January; became steadily high from February to May; a drop occurred in July after which there was another rise in August and October. For the females, there was no drop in the early part of the breeding season and this did not occur in the males of this population until May.

Aurs Burn

The males in this population showed no significant variations with months in their percentage stomach fullness although this was higher in April. In the females, there was a gradual increase from February to peak levels in April and May. A gradual decrease followed till the lowest levels in October. Females and possibly males of this population had their highest food intake levels at the early part of the breeding season and this was more marked in females.

Table 4.3 Summary of months for which percentage stomachfullness was significantly different: River Luggie.

Months	Sexes
April and November ($p=.03$)	Males
April and December ($p=.01$)	
April and January ($p=.01$)	
April and March ($p=.03$)	
August and November ($p=.01$)	
August and December ($p=.00$)	
November to January and February to May ($p=.01$)	Females
July and February to May ($p=.00$)	
July versus August and October ($p=.01$)	

Table 4.4 Summary of months for which percentage stomach fullness were significantly different: Aurs Burn.

Months	Sex
April and January ($p=.01$)	Females
April and October ($p=.00$)	
April and December ($p=.02$)	
May and January ($p=.00$)	
May and October ($p=.00$)	
May and December ($p=.00$)	

Population effects

Table 4.5 shows the mean and standard error of percentage stomach fullness for males and females of the 3 populations as well as the populations with significant differences in stomach fullness. There were differences in percentage fullness between the populations for both males and females. Rivers Kelvin and Luggie were not different from each other comparing either their males or females. However both sexes of these populations had significantly fuller stomachs compared to fish from the Aurs Burn.

Sex effects

Taking the year's data as a whole, there were no effects of gender on percentage stomach fullness for any of the three populations. For certain months analysed separately, there were significant gender effects but these are very variable. There was a significant effect of gender in July for both Kelvin and Luggie populations. Females had fuller stomachs compared to males in the River Kelvin but the reverse was the case in the Luggie. In the Aurs Burn, there was a significant effect in May with females having a higher percentage stomach fullness compared to males.

Age effects

There were no effects of age either taking the year's data as a whole or considering the months with the most variable age structure.

Table 4.5 Means and standard error of percentage stomach fullness for the populations showing those with significant differences.

Population	Sex	Percentage Stomach fullness
Kelvin	Female	53.1 ± 3.4
Luggie	Female	48.1 ± 3.4
Aurs Burn	Female	37.3 ± 3.0
Kelvin	Males	52.1 ± 4.4
Luggie	Males	43.1 ± 3.4
Aurs Burn	Males	28.1 ± 3.0

** = P<.01

Size effects

Tables 4.6 to 4.8 show the mean and standard error of the average percentage stomach fullness for the different sizes of fish in the 3 populations. There was a significant effect of size on percentage stomach fullness on Kelvin males. Table 4.9 summarizes the sizes with significant differences for Kelvin males (those in size category 3 and 4 have low food intake). No effect was found for the Kelvin females and for fish of either sex from the River Luggie and the Aurs Burn. The effects of size on percentage stomach fullness on months with similar feeding patterns produced no effect for any of the three populations. Smaller females of the Kelvin tended to have lower food intake in March, but in November, the reverse was the case so the significance of this is not clear.

4.3.2 Percentage of fish with empty stomachs

Table 4.10 shows the percentage of fish with empty stomachs for the three populations across the months. This was generally high in autumn and winter and low the rest of the year except in the Kelvin where it was still relatively high in the period May to July.

Table 4.6 Mean and standard error of percentage fullness for the different sizes of fish in River Kelvin.

Size Categories	Females	Males
1	37.4 \pm 19.7	27.7 \pm 2.8
2	74.3 \pm 6.9	30.4 \pm 11.1
3	48.8 \pm 10.7	6.9 \pm 6.9
4	54.8 \pm 9.5	27.9 \pm 8.7
5	49.4 \pm 9.9	58.9 \pm 10.6
6	56.7 \pm 10.9	67.6 \pm 8.5
7	46.8 \pm 8	47.9 \pm 9
8	62.2 \pm 8.7	60.5 \pm 14.2
9	53.8 \pm 13.7	-

Table 4.9 Sizes with significant differences in percentage fullness in River Kelvin.

Sizes	Sex
3 and 5 (p=.02)	Males
3 and 6 (p=.00)	
3 and 8 (p=.04)	
4 and 5 (p=.03)	
4 and 6 (p=.00)	

Table 4.7 Mean and standard error of percentage fullness for the different sizes of fish in River Luggie.

Size Categories	Females	Males
1	4 ± 41	-
2	47.9 ± 13.5	31.3 ± 0
3	55.7 ± 11.6	54.8 ± 10.1
4	51.3 ± 11.5	57.9 ± 7.1
5	32.6 ± 6.0	35.5 ± 6.5
6	49.3 ± 6.5	41.0 ± 5.9
7	55.8 ± 7.6	31.2 ± 7.3
8	45.0 ± 0	7.7 ± 7.7
9	89.1 ± 0	39.3 ± 31.2

Table 4.8 Mean and standard error of percentage fullness for the different sizes of fish in the Aurs Burn.

Size Categories	Females	Males
1	-	-
2	29.4 \pm 16.7	-
3	38.4 \pm 7.3	28.8 \pm 7.9
4	24.1 \pm 3.7	27.2 \pm 3.1
5	44.2 \pm 6.9	27.8 \pm 5.6
6	51.3 \pm 6.5	37.5 \pm 7.6
7	39.0 \pm 9.7	23.9 \pm 8.5
8	23.1 \pm 10.1	16.9 \pm 12.3
9	23.8 \pm 6.4	-

Table 4.10 Percentage of fish with empty stomach in the three populations.

Population		O	N	D	J	F	M	A	M	JN	JY	A
Kelvin	No. of fish	21	20	21	20	20	20	20	20	18	20	18
	% of empty stomach	14.3	20	14.3	15	5	10	5	10	11	15	0
LUGGIE	No. of fish	20	20	20	20	19	20	20	19	-	17	19
	% of empty stomach	5	15	15	30	5.3	0	0	0	-	0	0
AURS BURN	No. of fish	20	20	20	20	18	0	0	0	-	0	4
	% of empty stomach	5	25	5	20	22	0	0	0	-	0	20

O to A represents October to August.

4.3.3 Diet Composition

Tables 4.11 to 4.13 show monthly diet composition for the three populations.

River Kelvin

Asellus, Gammarus and Cyclops were the predominant food types for the whole year, except in July when the occurrence of Cyclops was at a temporary low level. Chironomidae larva formed 10 to 20 percent of the diet from January to August only. They were not found between October and December. Algae, plant material invertebrate eggs and mineral material formed a relatively small proportion of the diet in almost all the months. Algae and plant material was absent in June, invertebrate eggs in August while mineral material were absent in May, July and August. Stickleback eggs were only taken in May and June and then in very small amounts (less than 3 percent). Molluscs were taken between May and August, occurring at the highest frequency in May and June compared to July and August. Ephemeroptera occurred very rarely and was not included.

River Luggie

Asellus and Gammarus were predominant food types throughout the year (up to 75 percent) except between May and August when there were in quite low levels. Cyclops was present at most times of the year, being quite common in April (22%), May and August but unlike in the Kelvin, it was absent in November and January. Occurrence of

chironomidae and other dipteran larva were low between October and December, quite high between January and April (20 to 30 percent); from May to August, they became the predominant food type (up to 50% of the diet). Invertebrate eggs, stickleback eggs and molluscs were absent throughout the year. Ephemeroptera were present in variable quantities ⁱⁿ the whole year.

Table 4.11 Percentage composition of the diet at different times of the year in fish from the River Kelvin.

Months	Food Types									
	1	2	3	4	5	6	7	8	9	10
October	37.6	21.9	0	6.3	9.5	3.3	0	9.4	0	15.6
November	43.8	28.1	0	3.3	3.3	6.3	0	0	0	15.6
December	29.4	41.2	0	2.9	8.8	8.8	0	0	0	8.8
January	27.7	21.3	21.3	2.1	8.5	12.8	0	0	0	6.4
February	29.1	27.3	9.1	5.5	7.3	5.5	0	0	0	16.4
March	35.5	20	8.9	20	8.9	0	0	0	0	6.7
April	28.8	31.1	15.6	2.2	2.2	4.4	0	0	0	15.6
May	20.7	24.1	22.4	0	5.2	1.7	1.7	12.1	0	12.1
June	29.4	14.7	14.7	5.9	0	2.9	2.9	11.8	0	17.7
July	52.2	3.5	20.6	0	10.3	3.5	0	3.5	0	7
August	24.5	30.2	20.8	0	5.7	0	0	7.6	0	11.3

1 = Asellus and Gammarus; 2 = Cyclops; 3 = Chironomidae and other dipteran larva; 4 = Mineral material; 5 = Algae and plant material; 6 = Invertebrate eggs; 7 = Stickleback eggs; 8 = Mollusc; 9 = Ephemeroptera; 10 = Unidentified material.

Table 4.12 Percentage composition of the diet at different times of the year in fish from the River Luggie.

Months	Food types									
	1	2	3	4	5	6	7	8	9	10
October	58.4	16.6	5.6	2.8	0	0	0	0	0	16.7
November	31.8	0	13.7	0	4.6	0	0	0	0	50
December	50	7.7	11.5	0	0	0	0	0	0	30.8
January	35	5	30	0	0	0	0	0	0	30
February	38	3.5	24.1	0	3.5	0	0	0	0	31
March	47.5	12.5	20	2.5	2.5	0	0	0	0	15
April	35.9	22.6	26.4	1.9	1.9	0	0	0	1.9	9.4
May	11.1	31.1	37.8	0	2.2	0	0	0	4.4	13.3
July	6.7	13.3	50	0	0	0	0	0	10	20
August	10.5	23.7	34.2	2.6	0	0	0	0	2.6	26.3

1 = Asellus and Gammarus; 2 = Cyclops; 3 = Chironomidae and other Dipteran larva; 4 = Mineral material; 5 = Algae and plant material; 6 = Invertebrate eggs; 7 = Stickleback eggs; 8 = Mollusc; 9 = Ephemeroptera; 10 = Unidentified material.

Aurs Burn

The most important food items throughout the whole year were chironomidae and other dipteran larva (up to 65%). Asellus and Gammarus were present but very low throughout the year (never above 12%) they were completely absent in July and October. Cyclops was present in quite large amounts in the diet over the whole year especially between February and May (when it reached up to 28 percent). Algae, plant material and invertebrate eggs constituted relatively small amount in the diet almost the whole year while stickleback eggs were only present in May and then only in 3.6% of stomachs. Molluscs were eaten in quite large amounts in August, October and November, absent in February, April and May and consumed only in small amounts the rest of the year. Ephemeroptera was quite an important food item (22 percent) between January and April, absent in October and November and low the rest of the year.

Table 4.13 Percentage composition of the diet at different times of the year in fish from the Aurs Burn.

Months	Food Types									
	1	2	3	4	5	6	7	8	9	10
October	0	14.7	20.6	2.9	2.9	5.9	0	11.8	0	41.2
November	7.1	17.9	17.9	0	3.6	0	0	14.3	0	39.3
December	7.5	12.5	35	2.5	5	0	0	0	2.5	35
January	11.6	15.4	23.1	3.9	15.4	0	0	3.9	7.7	19.2
February	6.2	25	31.2	3.1	3.1	0	0	0	21.9	9.4
March	15	10	25	5	5	0	0	2.5	15	22.5
APRIL	10.9	28.3	26.1	0	2.2	0	0	0	23.9	8.7
May	1.8	27.3	32.7	0	0	0	3.6	0	7.3	9.1
July	0	22.7	36.4	2.3	4.6	11.4	0	2.3	11.4	9.1
August	5.3	15.8	34.2	7.9	0	2.6	0	21	7.9	5.3

1 = Asellus and Gammarus; 2 = Cyclops; 3 = Chironomidae and other dipteran larva; 4 = Mineral material; 5 = Algae and plant material; 6 = Invertebrate eggs; 7 = Stickleback eggs; 8 = Mollusc; 9 = Ephemeroptera; 10 = Unidentified material.

4.4 DISCUSSION

4.4.1 Stomach fullness

There was a definite seasonal rhythm in feeding activity in all three study populations. Feeding was generally high in the period before the breeding season followed by a drop at the early part of the season; for Luggie and Aurs Burn females this drop occurred late, percentage stomach fullness still being high in May. One possible explanation for the low food of males in the breeding season is that competition for territories and mates leaves little time for feeding.

Incontrast, Allen (1980) and Manzer (1976) observed high feeding levels during the breeding season. Although Hynes (1950) noticed a similar decrease in feeding at the beginning of the breeding season (which lasted from March to June in his population), he also found that feeding levels increased again when breeding was in full force. The increase observed in the present study did not occur until August in the Kelvin and Luggie fish and not at all for the Aurs Burn fish. Allen (cited above) also observed a feeding burst in August and attributed this to the influx of the young fish into the population. The same reason probably applies here, since the August sample was composed almost entirely of young fish and nearly all had full stomachs.

Food levels were low in winter for the three populations and this agrees with all the other studies (Hynes 1950; Manzer 1976; Allen and Wootton 1982). This could be due to low food availability in winter or to the fact that sticklebacks hunt their prey by sight so

short day lengths in winter would reduce the number of hours that could be spent feeding. It is not possible to distinguish between these possibilities, because food levels were not sampled in this study.

Manzer (1976) also reported a high percentage of fish with empty stomach in midsummer and low levels in spring, early summer and autumn. He suggested that the relatively high percentage of sticklebacks with empty stomachs in July was due to the presence of gravid females who were eating little food. Percentage of fish with empty stomachs in this study was in opposite direction to percentage fullness, low in spring and summer (except for River Kelvin) and high in autumn and winter.

The Aurs Burn population had the least food in their stomachs compared to the others; this again might be due to low food availability but there is no proof. Surprisingly, compared to the Kelvin fish, a smaller proportion of fish had empty stomachs throughout the year. It could be that the heavy stomach content of the Kelvin and Luggie sticklebacks is because their predominant food was the relatively large *Asellus* and *Gammarus* rather than the small chironomidae larva which was predominant in the diet of the Aurs Burn fish most of the year.

It appears that there are no simple and consistent relationships between age, size, gender and the percentage stomach fullness of the populations. Females had higher percentage stomach fullness than males in July and May for Kelvin and Aurs Burn populations respectively. In fish from the Luggie in July, the opposite was the

case and no clear explanations could be found for this. Both Allen (1980) and Manzer (1976) reported that males had higher feeding incidence throughout the summer.

4.4.2 Diet Composition

The most important food items in the Rivers Kelvin and Luggie were Asellus, Gammarus and Cyclops. Chironomidae larva was also important at these sites at specific times of the year, but in the Aurs Burn they were the most important food type for most of the year. Asellus and Gammarus were also present but in small amounts. This agrees with Maitland's 1965 study of the food of fish in River Endrick, Scotland, where he found the main food of the leiurus sticklebacks to be Gammarus and Asellus. Hynes (1950) study also included the food of the trachurus form of sticklebacks in a brackish water in Easdale Island in Scotland and he also showed their main food to be Gammarus. Investigating the food of the leiurus sticklebacks in Bare stream in Dorset, England, Mann and Orr (1969) found they were consuming a large amount of crustaceans such as Gammarus and Asellus as well as many insect larva. Both Manzer (cited above) and Allen (1980) reported copepods, cladocerans and chironomidae larva as the main food of sticklebacks in their studies. Hynes (cited above) found a predominant insect diet (aquatic larvae) for the three spined sticklebacks during the summer and an increasing importance of crustaceans in the winter.

Manzer (1976) noted that for two species of cladocerans, their changes in abundance in the lake were reflected in the changes in their proportion in the diet of the sticklebacks. He and several

other authors have therefore suggested that seasonal changes in the diet of sticklebacks are caused by changes in the density or availability of the prey rather than by changes in the preferences of the sticklebacks. The diet of sticklebacks observed in the present study probably reflects what was available to them in the wild. Cannibalism (eating of stickleback eggs) was relatively low in the present study compared to others (such as Hynes 1950, Manzer 1976, Whoriskey and FitzGerald 1985b). The differences in temperature at different times of the year may have been responsible for differences in food intake although Allen (1980) concluded that temperature did not correlate with fluctuations in food consumption, but that measurements of the diel variations in stomach content as well as the rate of digestion were necessary in showing seasonal patterns of change in food of sticklebacks.

This chapter has shown that percentage stomach fullness was low in winter and high just before the breeding season commenced. Low food intake in winter has been shown for many other stickleback populations (Hynes 1950, Manzer 1976 and Allen 1980). No relationship was found between age and food intake and the relationship with gender of the fish was not consistent. Asellus and Gammarus formed the highest proportion of the food of sticklebacks from Rivers Kelvin and Luggie while chironomid larvae was the food item taken mostly by fish from the Aurs Burn, which also had lowest stomach fullness.

CHAPTER 5

GONADAL DEVELOPMENT AND ^{ITS} RELATIONSHIP [^] TO LENGTH AND WEIGHT

5.1 INTRODUCTION

5.1.1 Somatic and gonadal growth in fish

Literature discussed in chapter 1 suggests that in some fish, gonadal development occurs at the expense of somatic growth. The study on the gonadal development is described in the present chapter; accompanied by a consideration of its relationship to length and weight of the fish.

Many theoretical treatments of life history evolution assume that different life history components are inversely related, an increase in a particular variable being associated with a decrease in another. Gadgil and Bossert (1970) for example assumed that an increase in the resources devoted to reproduction resulted in decreased growth and survivorship. Many studies have been based on the idea of such a trade-off between gonadal and somatic growth. Reznick (1983), studying the somatic growth of different stocks of Trinidad guppies (that differed genetically in the amount of energy they devote to reproduction), found that increases in the amount of energy devoted to reproduction were matched by decreases in the energy devoted to somatic growth. Hirshfield (1980) working on the Japanese medaka found a negative correlation between somatic growth and energy devoted to reproduction among individuals within a group. Wootton (1977) found similar results for female sticklebacks. This raises the possibility that different rates of growth of the 3 populations used in the present study might be the result of some sort of trade-off between gonad and somatic growth.

Fecundity of female sticklebacks during the breeding season is dependent on food supply (Wootton 1973) and for the males, low food supply has been shown to inhibit breeding activities such as nest construction, Stanley and Wootton (1986). As shown in chapter 4, the 3 populations differed in their percentage stomach fullness, fish of both sexes from the Rivers Kelvin and Luggie having higher percentage fullness compared to the Aurs Burn population. Differences in food consumption might therefore have an effect on growth and or gonadal development in the present study.

5.1.2 Gonadal development in sticklebacks

Considerable attention has been given to the gonadal development of sticklebacks in the past, but few of these studies have been fully quantitative. Swarup (1958) divided the development of the germ cells into three stages: the period of undifferentiated germ cell, the period of primary sex differentiation of the germ cells and the period of development of the ovaries and testis.

The thin visceral peritoneum which covers the testis contains many melanophores, so that in contrast to a female ovary, a male's testis are pigmented black. Spermatozoa develop from meiotic division from spermatogonia to spermatocytes to spermatids and then to a mature sperm. A ripe sperm has a flattened disc shaped head with a distinct middle piece and a tail used for propelling the spermatozoa along (Swarup, 1958). In the fully mature gonad characteristic of the breeding season from mid April to early August (Craig-Bennett 1931), the seminiferous tubules are packed with spermatids and sperm; earlier stages are difficult to find and the interstitial tissue,

which secretes the gonadal hormones, is well developed, conspicuous and occupies wide areas (Ahsan and Hoar 1963).

Tromp-Blom (1959) distinguished three layers making up the wall of the ovary of the female sticklebacks; an outer peritoneum, a middle of thick connective tissue and an innermost layer of germinal epithelium from which the new ova arise. He also distinguished the following stages in the development of a young oocyte into a mature ovum:

Stage 1 : The nucleoli are very distinct and placed along the periphery of the nucleus.

Stage 2 : The nucleoli become dispersed over the nucleus

Stage 3 : Vacuoles start to form in the cytoplasm.

Stage 4 : The whole cytoplasm is filled with vacuoles.

Stage 5 : Yolk begins to form in the cytoplasm.

Stage 6 : This is the mature ovum with a large amount of yolk surrounded by a small layer of cytoplasm.

The oocytes do not all develop at the same time, so that just prior to spawning in spring, the ovary contains oocytes in all phases of development (Tromp-Blom 1959). Wallace and Selman (1979) studying oogenesis in female sticklebacks however found that oocytes enter maturation or yolk deposition as a group. Once a set of oocytes begin maturation, a new clutch is recruited into the early stages of yolk deposition or vitellogenesis. Thus they suggested that Tromp-Blom's original comments should be amended to say that immediately after deposition of eggs, all stages are present up to the early stages of vitellogenesis; mature eggs do not "gradually increase in

numbers". Immediately after the first spawning, mature ova are absent from the ovary but most stages of the oocyte are still present with the early developmental stages predominating (Stanworth 1953). Baggerman (1957, 1972) points out that maturation in sticklebacks is attained only after a period of exposure to low temperatures and short day length. She and Craig-Bennett (1931) also both indicated that the environmental factors triggering maturation; light and temperature are effective only for fish above a critical minimum body size. It has been suggested that this combination perhaps prevents individuals hatched early in the spring from spawning until the next spring, even though they have reached the critical size for maturation in their first summer.

The results discussed in chapter 3 suggest that the breeding season is longer than in most previously studied stickleback populations, and that late hatched fish may not mature to breed at the next season following birth. Data were therefore collected on gonad development across the year in fish of different sizes to clarify patterns of growth and the implications for value of broods at different times of the breeding season. The work described in this chapter therefore had the following aims:

- (1) To extend existing information by collecting further quantitative data on gonad size and development and how relative development of the different gonadal stages vary across the year.
- (2) To clarify the position of late hatched fish by finding out if different age and size classes of sticklebacks differ in size and relative development of their gonads.

- (3) To find out if the study populations differ in gonad weight and development and if so whether this can be related to body size.
- (4) To find out how body condition varies across the year and to relate body condition to gonadal development; in particular, to see whether gonadal growth occurs at the cost of body condition.
- (5) To find out how body condition and gonadal development relate to food supply.

5.2 MATERIALS AND METHODS

This analysis was carried out on the fish collected for characterization of population age structure (chapter 3). After the 20 fish had been measured and weighed, the gonads were dissected out, weighed and preserved in 10% formalin for histological analysis. All sections were cut at 7 μ m and stained with Haemalum and Eosin.

For the ovary, a count was made of the number of eggs in the different stages as distinguished by Tromp-Blom (1959). To turn frequencies into a measure of contribution to overall ovary size, a conversion factor was introduced which involved multiplying the proportion of eggs in each stage by their average area, based on the radius of 20 cells. Five fields of view for each fish under the microscope was found to give an accurate picture of the developmental stage of the ovary, because percentage of the different stages became constant with five fields of view. Plates 5.1 to 5.3 show examples of the different stages of ovary development while Plate 5.4 shows the ovary of a post-reproductive female.

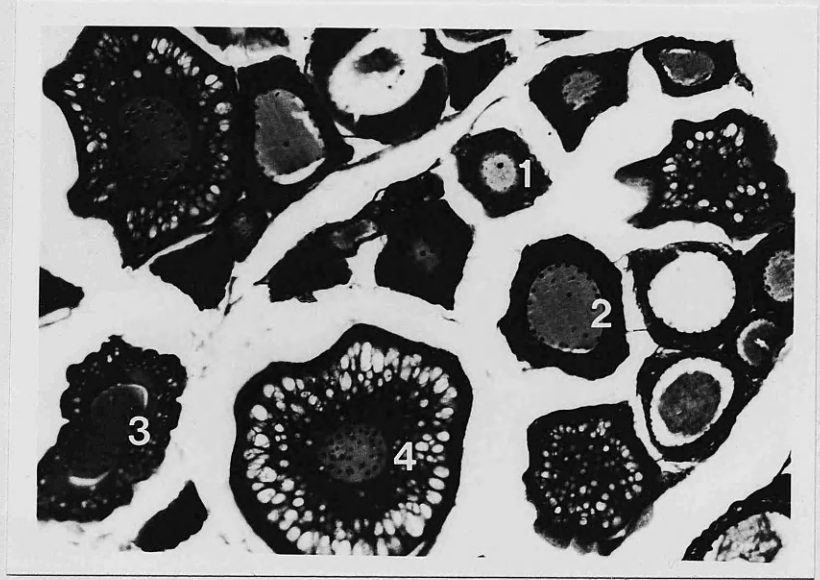


Plate 5.1 Section of the stickleback ovary showing the first 4 stages of ova development (x160). 1 = Stage 1, 2 = Stage 2, 3= Stage 3, 4 = Stage 4.

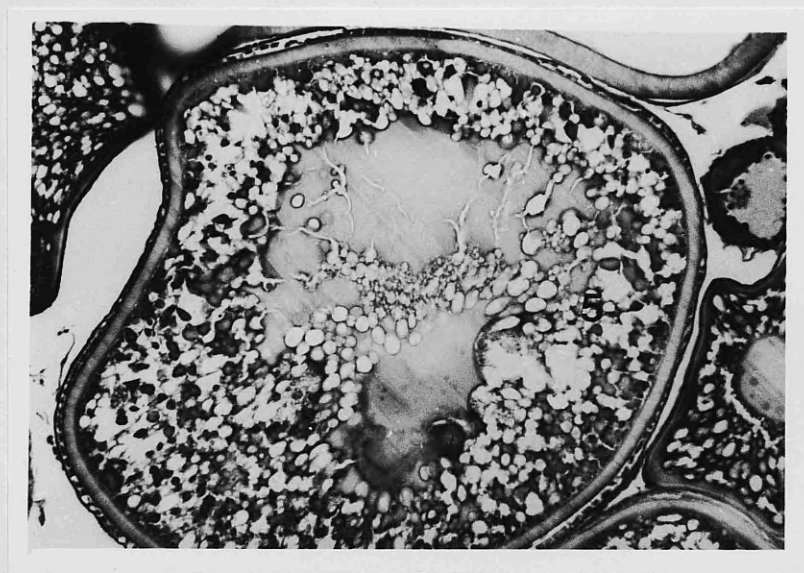


Plate 5.2 Section of the stickleback ovary showing stage 5 (x160)

5 = Stage 5

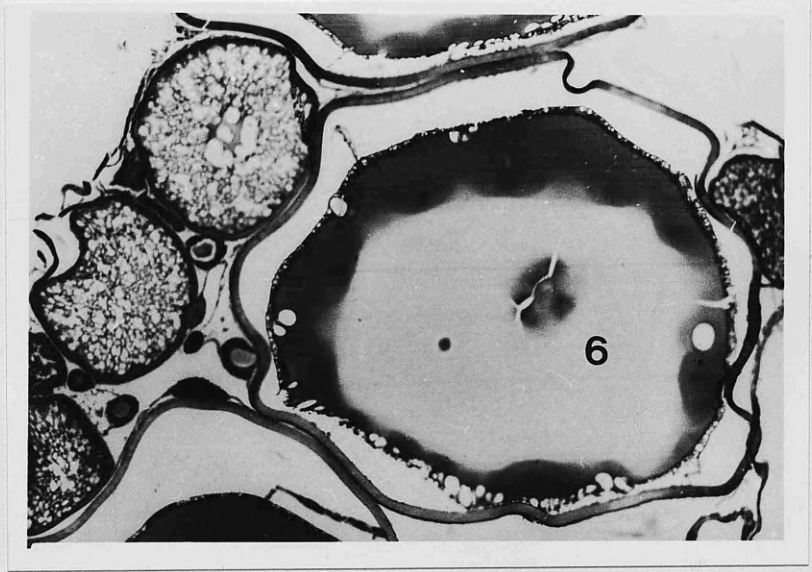


Plate 5.3 Section of the stickleback ovary showing stage 6 (x65)

6 = Stage 6



Plate 5.4 Section of the stickleback ovary showing collapsed tissues
(x160)

For the testis, a set of 25 points at random was placed in the eye piece of the microscope and the number of these points that fell on cells in the different stages of spermatogenesis were recorded. Again five fields of view was used since this produced consistent percentage values. Because of the difficulty of distinguishing between primary and secondary spermatocytes without the use of an electron microscope (Scott, pers. comm.), these two stages were regarded as one in the analysis of the testis. Plates 5.5 to 5.6 show examples of the four stages of sperm maturation. Plate 5.7 shows testis of a post-spawning, male with empty tubules. In each gonad, the total number of points that fell on the various stages was summed up and the proportion of each stage was calculated as a percentage of this total.

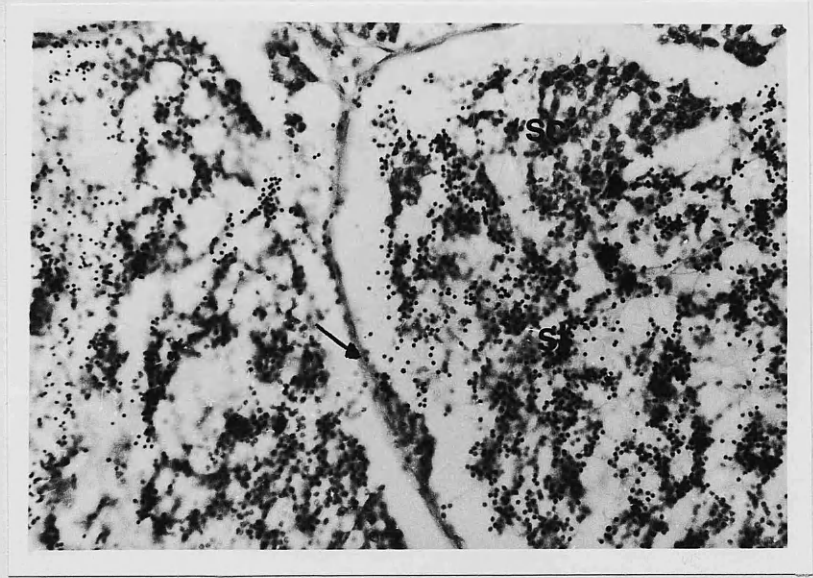


Plate 5.5 Section of the stickleback testis showing spermatogonia (arrow), spermatocytes (sc) and spermatids (st) (x390)



Plate 5.6 Section of the stickleback testis showing spermatozoa throughout (x390)

The gonad weight/body weight ratio of all fish was calculated to provide a single index of gonadal development for later analysis. A saturation curve was also calculated for all fish. In females this was by using a range of 0.001 to 0.01 g/g and for males, the spermatid and spermatozoa were used. A gonadosomatic index (GI) was calculated using the formula: $GI = \frac{G}{W} \times 100$ (Parr and Mills 1959).

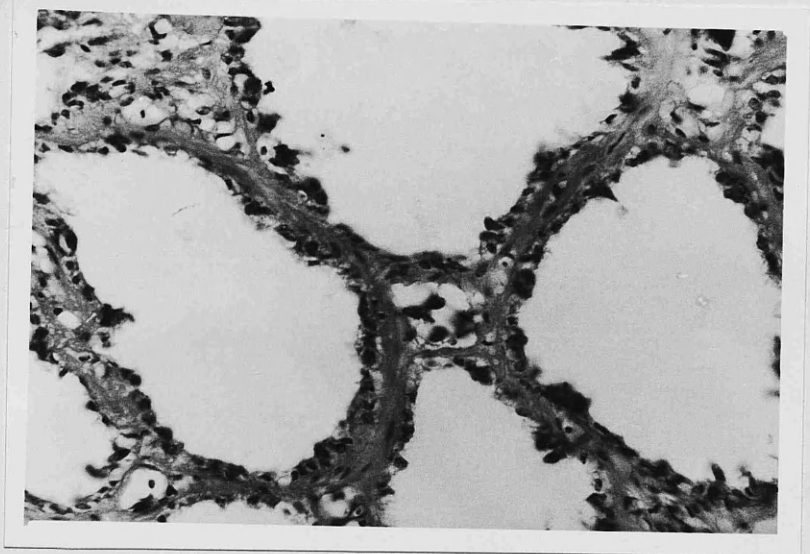


Plate 5.7 Section of the stickleback testis showing a post spawning male with empty tubules (x390)

The gonad weight/body weight ratio (gw/bw) of all fish was calculated. To provide a single index of gonadal development for later analyses, a maturation score (MS) was also calculated for all fish. In females this was by adding stages 5 and 6 together and for males, the spermatids and spermatozoa was used. A condition factor (CF) was calculated using this formulae (adapted from Wootton, Evans and Mills 1978):

$$CF = \frac{\text{Body weight} - \text{Gonad weight}}{(\text{Length})^3}$$

*

5.2.1 Data analysis

Seasonal changes in gonadal and body condition

One-way analysis of variance was used to check for seasonal variations in gonad weight/body weight ratio, maturation score and condition factor. Their mean and standard error is plotted so that significant differences can be assessed.

Differences between populations

One-way analysis of variance was used to check for differences between populations in body length and weight, gonad weight, gw/bw ratio, maturation score and condition factor. When a significant difference was found between populations, a two sample t-test was used to check where the difference lies.

Differences between fish of different age groups

Differences in gonad weight/body weight ratio and in maturation score was looked at using one-way analysis of variance. Effects on all months pooled together was looked at first; then to allow for seasonal effects months with the most variable age structure were chosen and tested together. These months were October to December for River Kelvin, October and December of the Luggie and October, November and August for the Aurs Burn.

Association between the body features (length and condition factor) and gonadal development measures (gw/bw ratio and maturation score)

This was looked at using a simple regression analysis. Gw/bw ratio and maturation score were used as the dependent variables with length and condition factor as the independent variables in this test and fish of different age groups were treated separately.

Association between stomach fullness and the following parameters: length, weight, gonad weight, gw/bw ratio, condition factor and maturation score

A simple regression analysis was used to test for any relationship between stomach fullness and length, weight, gonad weight, gw/bw ratio, condition factor and maturation score.

5.3 RESULTS

5.3.1 Seasonal changes in gonadal condition

Gonad weight/body weight ratio

Figs. 5.1 and 5.2 show plots of gw/bw ratio against months for females and males of the 3 study populations.

Females of all 3 populations showed a significant variation in gw/bw ratio across the months. They showed a peak before and during the breeding season, though this was much more marked in the Kelvin and Aurs Burn females than in the Luggie females, who had a lower ratio throughout the year.

The River Kelvin males alone varied significantly in their gw/bw ratio over the year, levels in the winter months being very slightly higher. Generally, gw/bw ratio stayed more or less constant across the year.

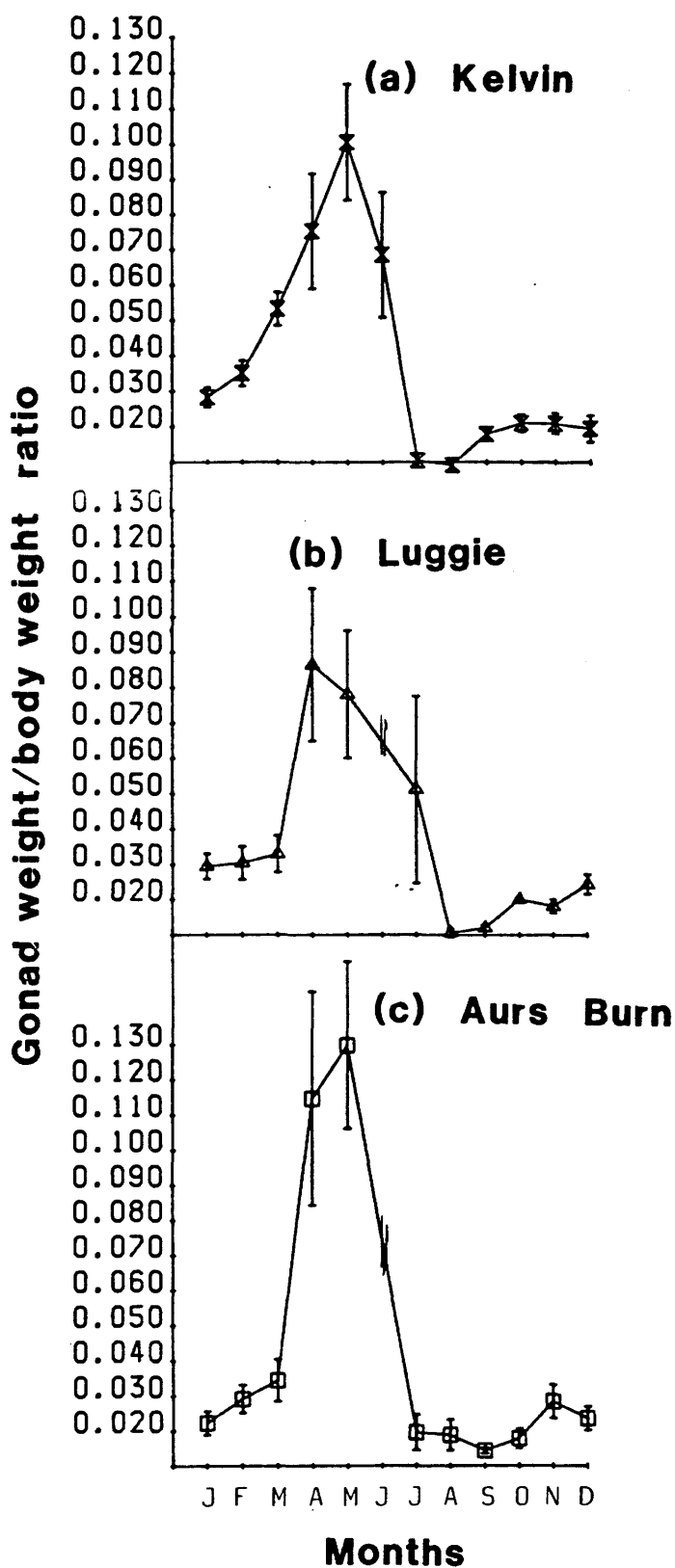


Figure 5.1 Mean and standard error of gonad weight/body weight ratio against months for females of the three populations.

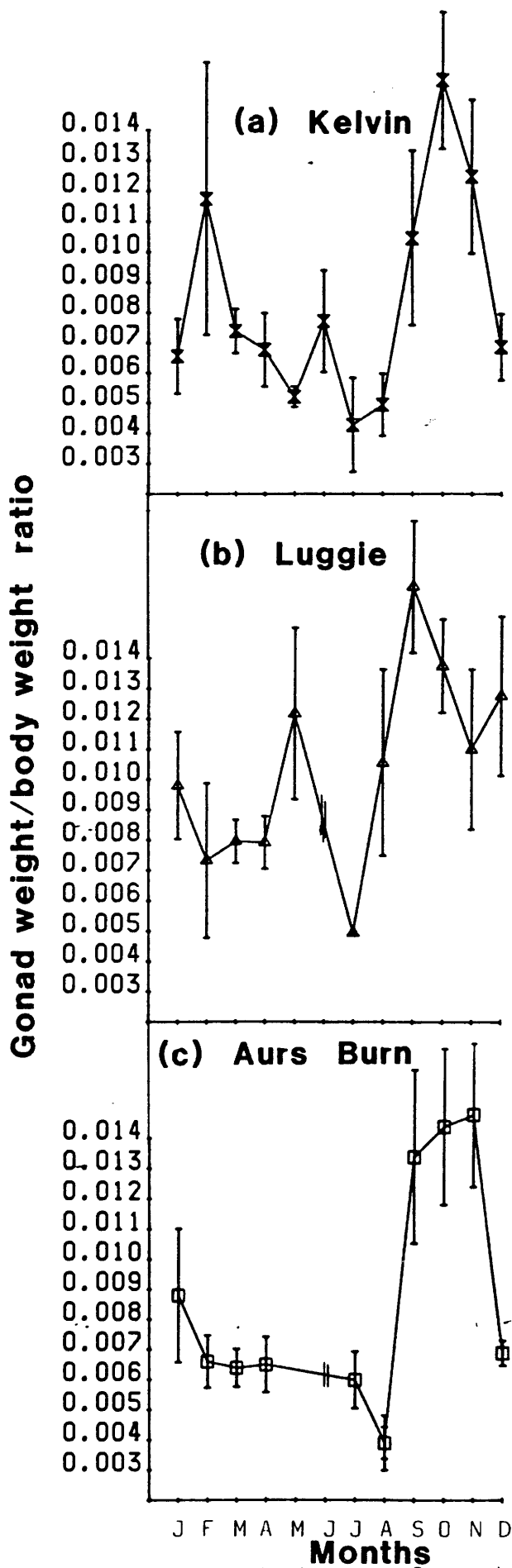


Figure 5.2 Mean and standard error of gonad weight/body weight ratio against months for males of the three populations.

Ovarian development

Figs. 5.3a, 5.4a and 5.5a shows plots of different stages of ovarian development for the three populations.

Stage one for all females of the 3 populations was highest in the period between July and December. The second stage was almost the same the whole year for the Luggie and Aurs Burn fish. In the Kelvin, it achieved higher but variable levels between July and December. Stage three was high most of the year except between July and September for all females. Stage four was observed in quite large amounts as the fish was coming into breeding between February and May after which there was a general decline. Stage five was first observed in March in Kelvin populations and in April in the other two populations. It increased as the breeding season progressed, dropping back to low levels towards the end of the breeding season in July and August. Stage six was first observed in April for the 3 populations and the highest levels were seen in May for the Aurs Burn and Kelvin females and in April for the Luggie. Stage 6 then declined in frequency until the end of the breeding season in August for all the 3 populations.

Testis development

Figs. 5.3b, 5.4b and 5.5b show plots of the stages in testis development for the three populations.

Spermatogonia were found in very small numbers across the year but tended to be absent at the peak of the breeding season between May

and July. Spermatocytes were commonest in the period between July and December (when the young of the year had been recruited into the population) and occurred at lower levels for the rest of the year. In contrast, the spermatids were most abundant between January and June as the males were reaching maturity and coming into breeding condition. The annual pattern of occurrence of spermatozoa differed to a surprising degree between populations. Spermatozoa could be observed in large amounts in Aurs Burn males as early as December and remained comparatively high until May. In the Kelvin, the high levels were found between April and August and for the Luggie, spermatozoa was only present in the period between April and June.

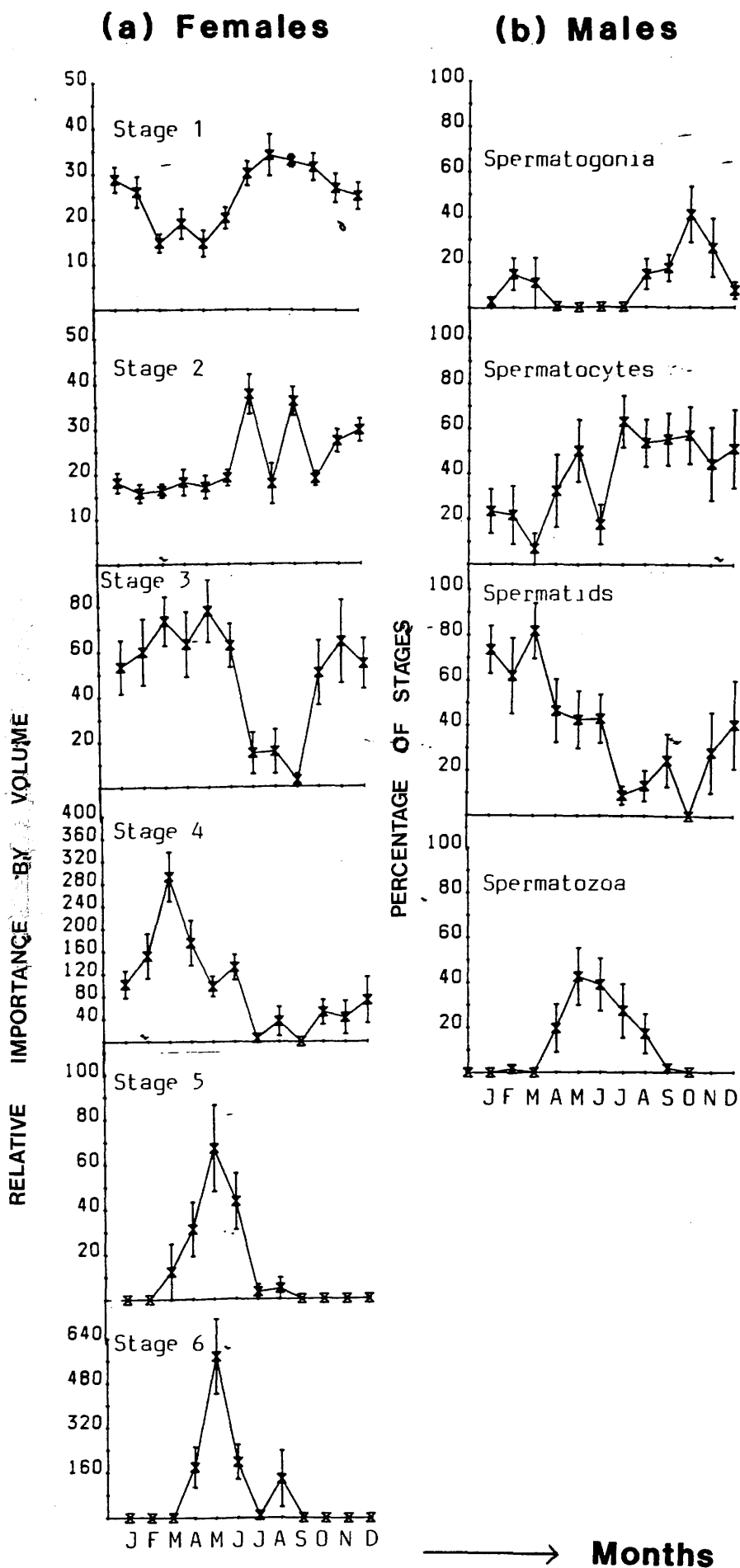


Figure 5.3 Mean and standard error of percentage of the stages of gamete production in fish from River Kelvin.

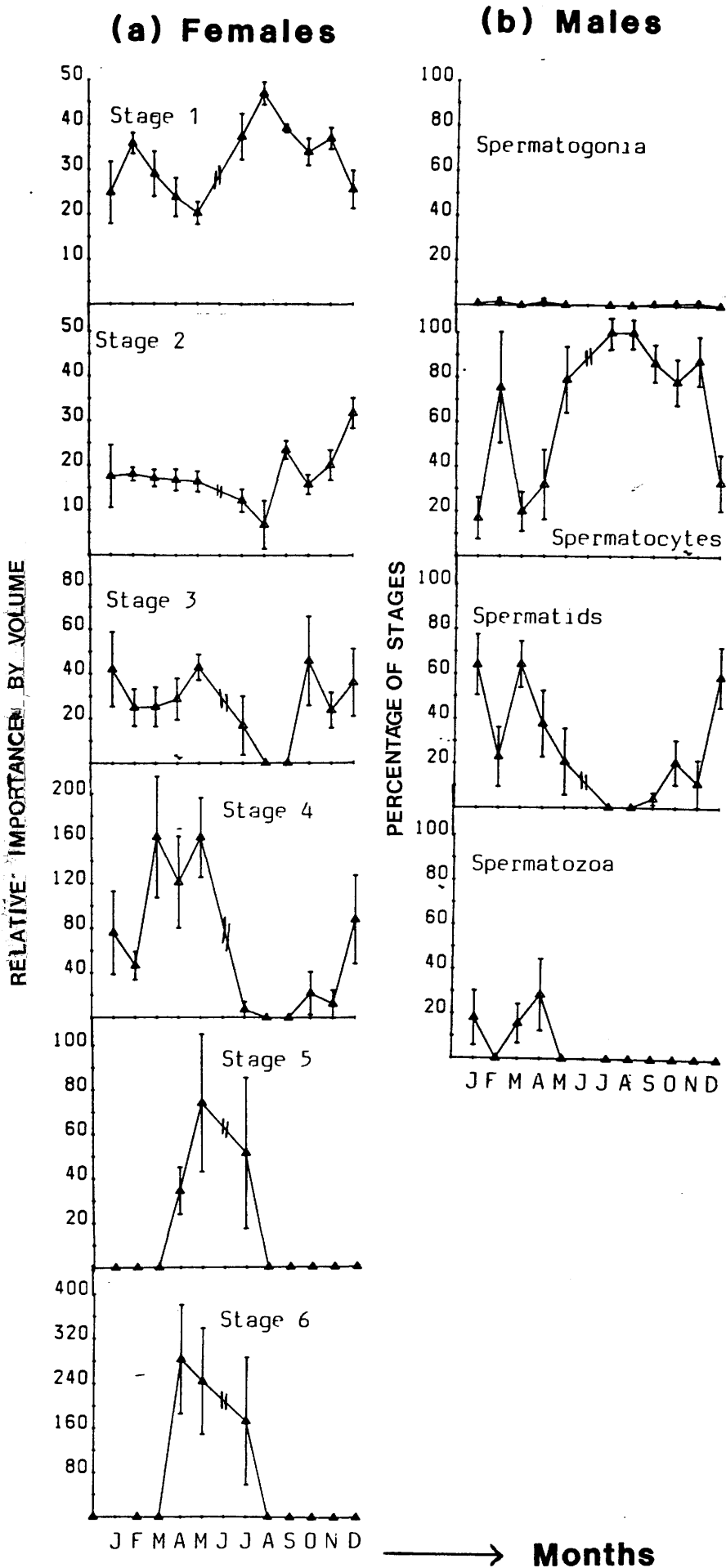


Figure 5.4 Mean and standard error of percentage of the stages of gamete production in fish from River Luggie.

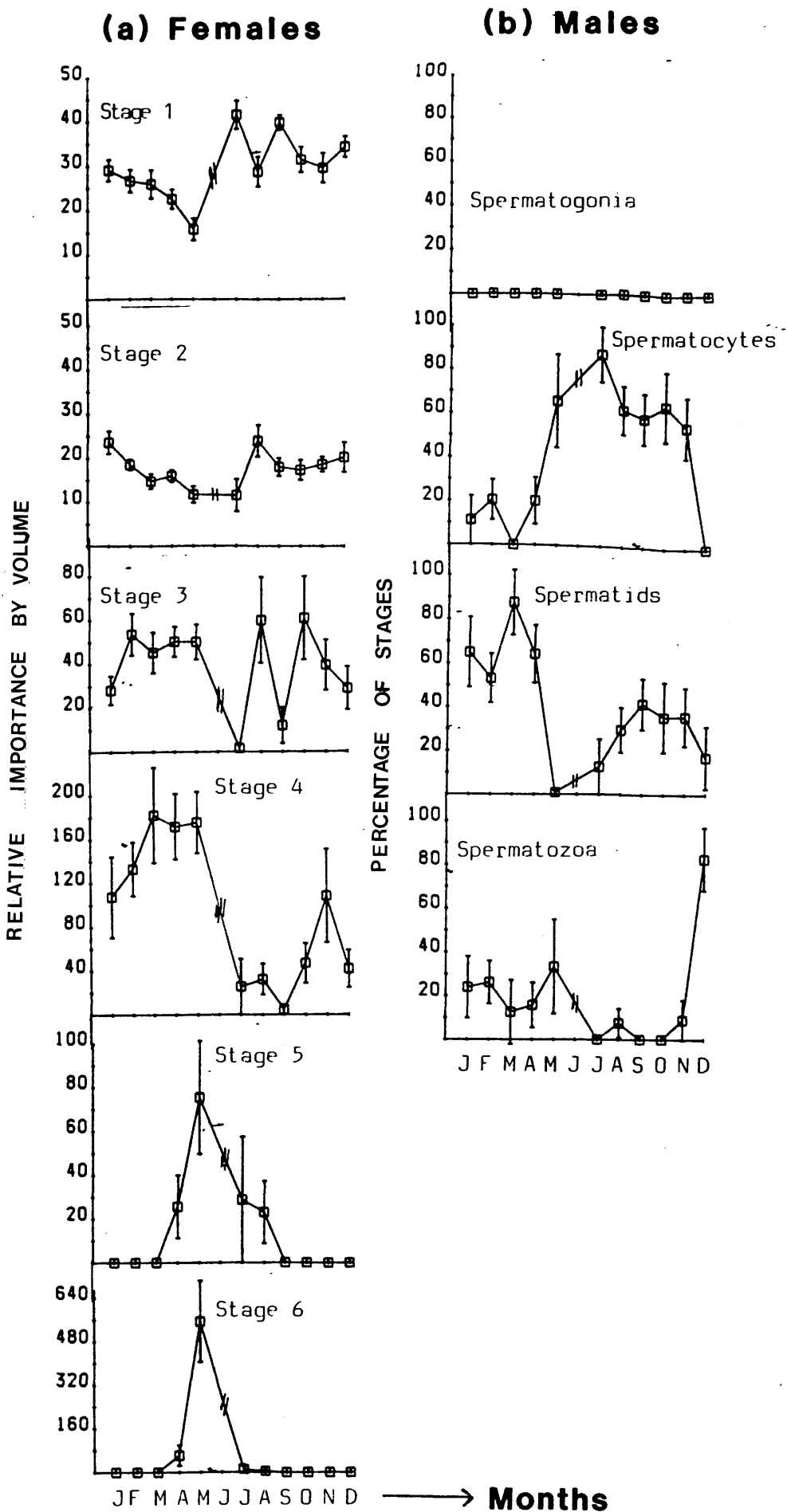


Figure 5.5 Mean and standard error of percentage of the stages of gamete production in fish from Aurs Burn.

Maturation score

Figs. 5.6 and 5.7 show plots of maturation score against months for females and males of the study populations.

There was significant variation in maturation score over the year for females of the 3 populations. In all females the highest maturation score occurred during the peak of the breeding season, from April to July. It was highest in May for Kelvin and Aurs Burn females and in April and May for the Luggie females. Males of all three populations showed a significant variation across months in their maturation score. Maturation score was high between January and April for Luggie and Aurs Burn males, and high up to June for Kelvin males. There was then a decline but levels rose again in September for the Aurs Burn males and later for the other two populations.

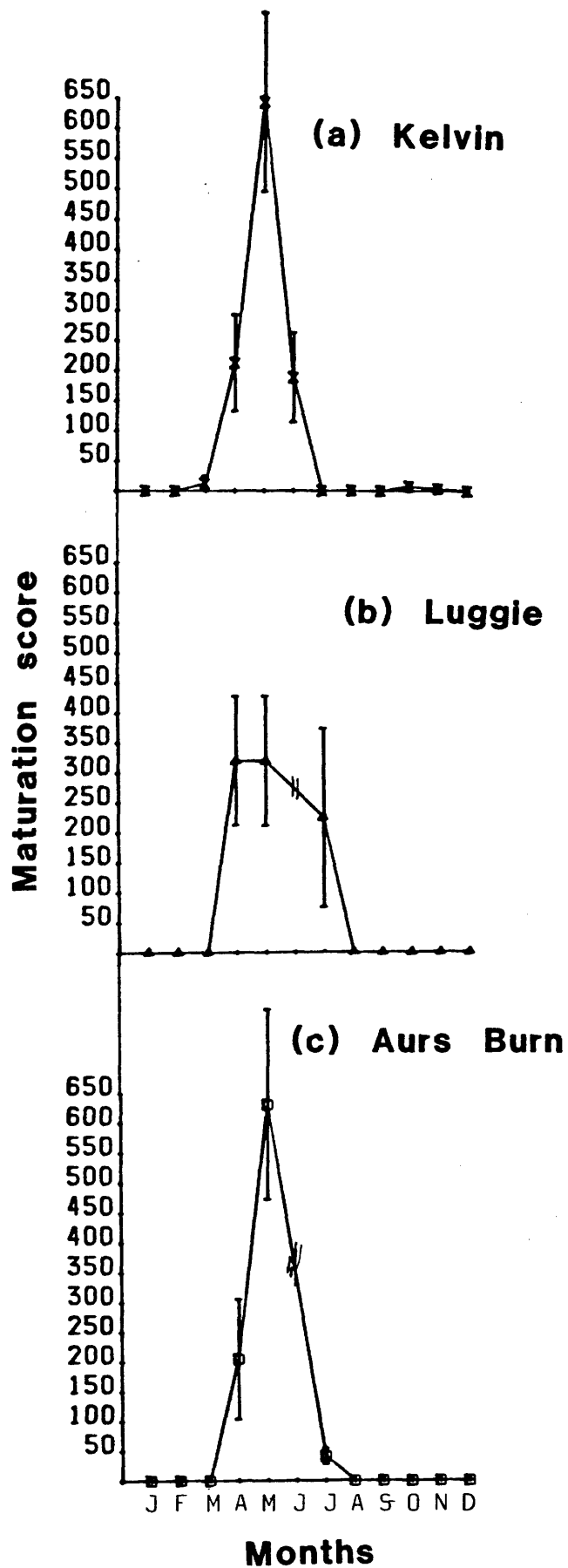


Figure 5.6 Mean and standard error of maturation score against months for females of the three populations

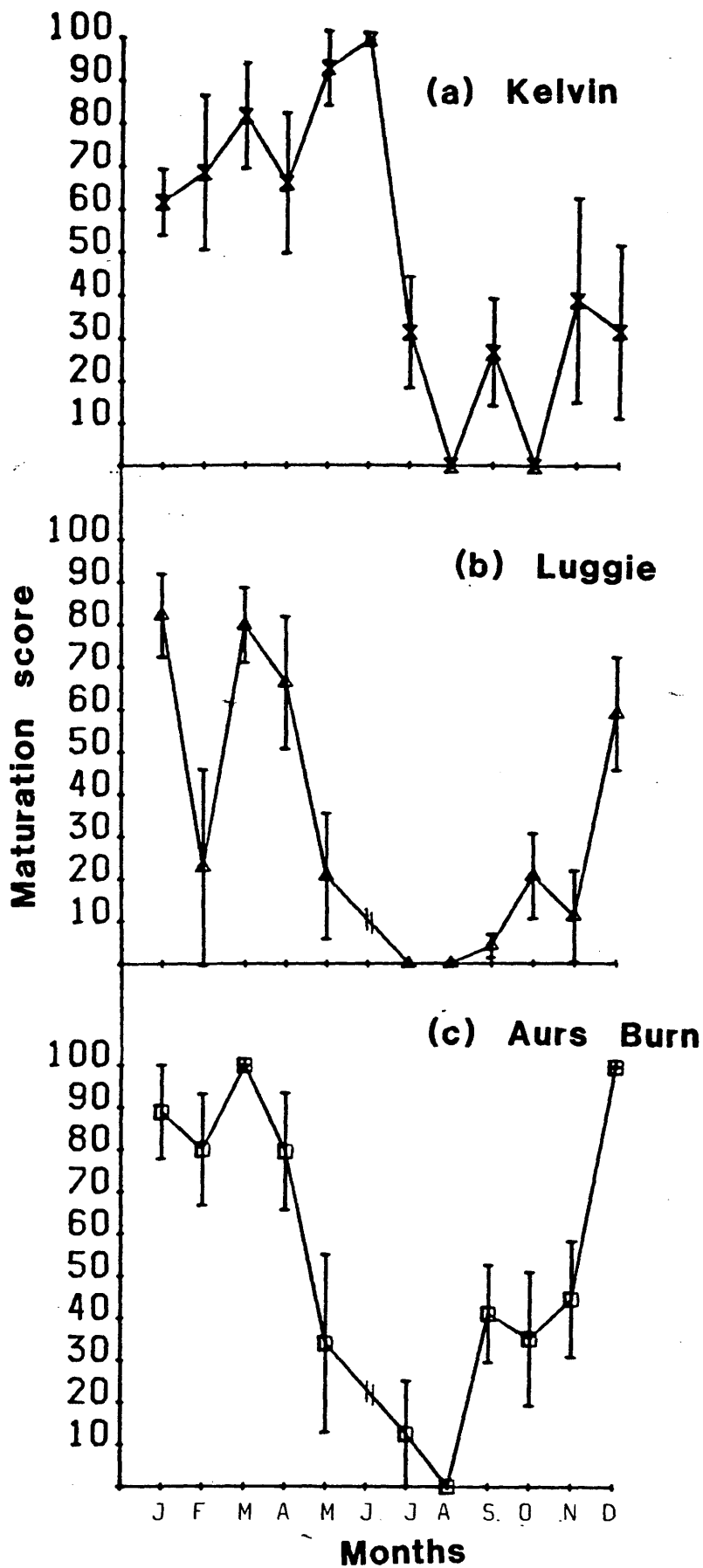


Figure 5.7 Mean and standard error of maturation score against months for males of the three populations.

Condition factor

Figs 5.8 and 5.9 show plots of condition factor against months for females and males of the 3 study populations. There was significant variation in condition factor across months for males of all 3 populations. A significant difference in condition factor across months occurred for Kelvin and Aurs Burn females but not for the Luggie females. For the females there was a rise in condition factor in April and May for the Luggie and Aurs Burn population and in March and April for the Kelvin. Then followed a decline but in Kelvin and Luggie fish, there was another rise in August though this was more marked in the Kelvin. Kelvin males had a high condition factor most of the year except in September when condition factor was low. Luggie and Aurs Burn males had high condition factor during the breeding season between April and August and lower levels the rest of the year.

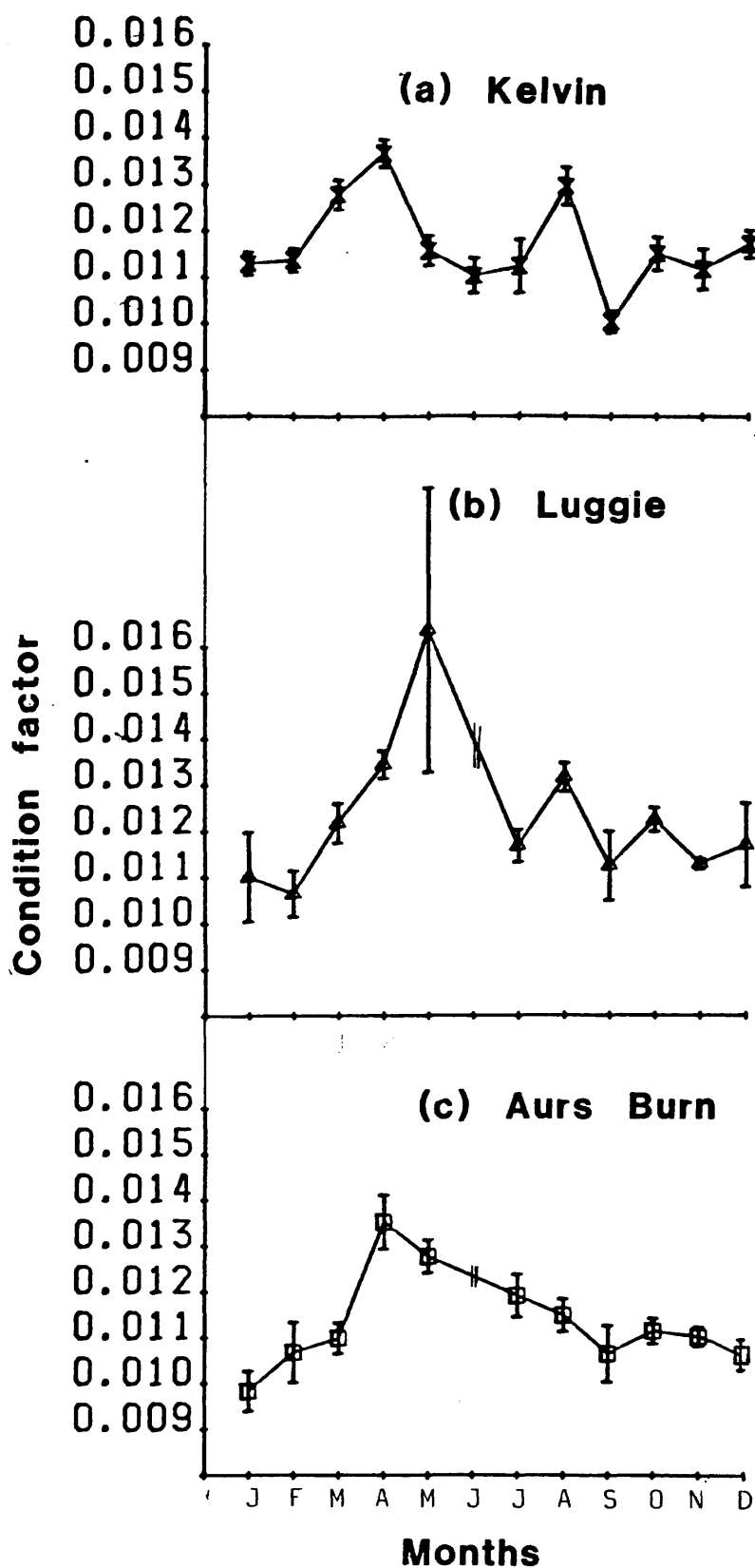


Figure 5.8 Mean and standard error of condition factor against months for females of the three populations.

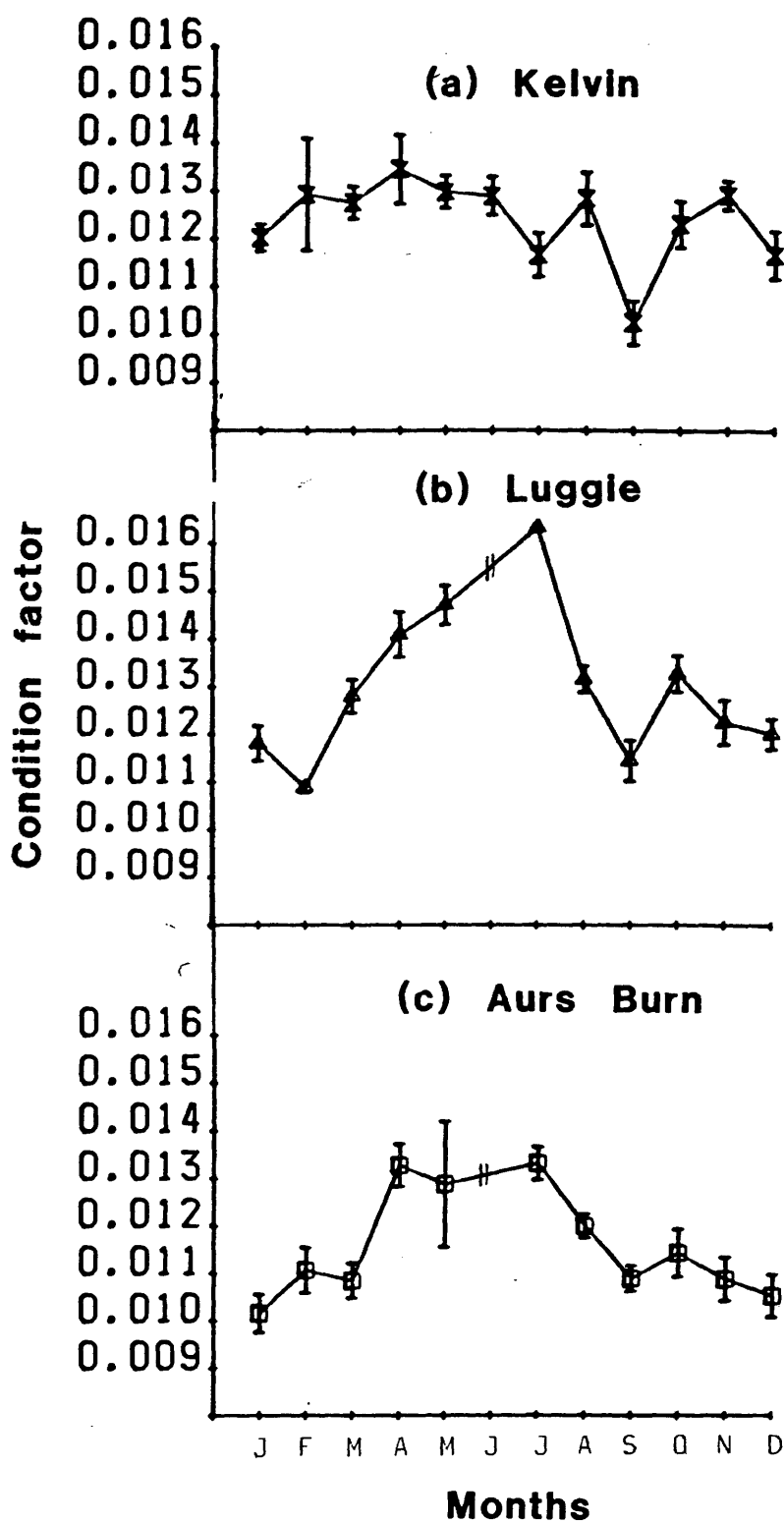


Figure 5.9 Mean and standard error of condition factor against months for males of the three populations.

5.3.2 Differences between populations

Table 5.1 shows means and standard error of the various parameters measured in the three populations, together with those with significant differences.

Analysis of variance indicated that there were no significant population effects for gonad weight or gw/bw ratio for either males or females. On the other hand, significant population effects were found for length ($p < .05$ for males; $p < .01$ for females), weight ($p = < .05$ for males; $p < .01$ for females), condition factor ($p \leq .01$ for males; $p < .05$ for females) and in the case of males, for maturation score ($p < .05$).

A two sample t-test showed that the Kelvin and Aurs Burn females were significantly longer and heavier than the Luggie females. The Luggie females however had higher condition factor compared to the other two populations but this difference was only significant when they were compared with the Aurs Burn females.

For the males, fish from the River Kelvin population attained the greatest length and weight compared to the other two populations. This difference was significant for the Luggie males (in length) and for both populations in weight. The Aurs Burn males however had the highest maturation score but they were only significantly different from the Luggie males. Luggie and Kelvin males had a significantly higher condition factor than the Aurs Burn males.

Table 5.1 Means and standards errors of different parameters for the populations showing those with significant differences.

Sex	Population	Length	Weight	G. W.	Gw/Bw	MS	Cf
F	Kelvin	4.260 \pm .076	1.089 \pm .059	.059 \pm .0072	.0390 \pm .0032	185 \pm 26.3	.01162 \pm .00013
F	Luggie	3.673 \pm .086	.763 \pm .061	.042 \pm .0074	.0386 \pm .0048	155 \pm 30.8	.01230 \pm .0043
F	Aurs Burn	4.094 \pm .085	.956 \pm .056	.062 \pm .0104	.0455 \pm .0049	190 \pm 32.9	.01135 \pm .00017
M	Kelvin	4.138 \pm .065	.952 \pm .042	.0077 \pm .0006	.0081 \pm .0006	54.1 \pm 4.1	.01236 \pm .00017
M	Luggie	3.878 \pm .074	.824 \pm .053	.0089 \pm .0007	.0112 \pm .0007	42.9 \pm 4.8	.01266 \pm .00017
M	Aurs Burn	4.007 \pm .064	.799 \pm .035	.0095 \pm .0032	.0108 \pm .0027	59.7 \pm 4.1	.01150 \pm .00019

M = Males; F = Females; Kelvin: Males, N = 121; Females, N = 167; Luggie: Males, N = 89; Females, N = 98; Aurs Burn: Males, N = 122; Females, N = 147. N = Numbers; ** = $p < .01$.

5.3.3 Gonad weight/body weight ratio and maturation score in fish of different age groups

Table 5.2 and 5.3 show mean and standard error of gw/bw ratio and maturation score for females and males of different age groups of the 3 study populations. Differences between age groups were first tested for the whole year together, then months with variable age structure were chosen and the same test was repeated to allow for any confounding effects of season.

There is a clear relationship between age and gonadal development in the females of the three study populations as reflected by both gw/bw ratio and maturation score. This was less robust in the Luggie females where no significant effect of gw/bw ratio and maturation score versus age was obtained when months with variable age structure were tested.

Paired t-test showed that for the Kelvin females, the older fish had significantly higher gw/bw ratio and maturation score compared to the younger ones. This was also true for Luggie females when the whole year was tested together. Older Aurs Burn females also had higher gw/bw ratio and maturation score but the difference was only significant comparing fish aged 0 and one year (two year old fish are rare).

In the males, there is less by way of a relationship between fish age and gonadal development, different results being obtained for the

different measures (gw/bw ratio and maturation score) as well as in the different populations. Males of different ages in the 3 populations did not differ in their gw/bw ratio. They however did differ in their maturation score (older fish having higher scores) in the Luggie and Aurs Burn (testing months with variable age structure) and in the Kelvin (testing the whole year); in each case, older fish had higher scores.

Table 5.2 Means and standard errors of gonad weight/body weight ratio and maturation score for females of
of different age groups of the three study populations, showing those with significant differences.

Sex	Population	Age	GW/BW ratio		Maturation Score	
			Whole Year	Month with variable age structure	Whole Year	Month with variable age structure
F	Kelvin	0	.0216 \pm .0017	.0163 \pm .0012	72 \pm 14	22.4 \pm 17.6
F		1	.0519 \pm .0056	.0307 \pm .0020	264 \pm 39.9	151 \pm 37.4
F		2	.1175 \pm .0089		1095 \pm 182.5	
F	Luggie	0	.0211 \pm .0015	.0215 \pm .0024	35 \pm 8.4	41.3 \pm 21.6
F		1	.0764 \pm .0124	.0225 \pm .0004	415 \pm 77.7	62.2 \pm 58.4
F	Aurs Burn	0	.0231 \pm .0016	.0129 \pm .0019	68 \pm 11.9	2.3 \pm 2.3
F		1	.0641 \pm .0088	.0293 \pm .0032	475 \pm 175.8	96.9 \pm 21.3
F		2	.1926 \pm .0694		526 \pm 291.8	
F = Females; ** = p< .01						

Table 5.3 Means and standard error of gonad weight/body weight and maturation score for males of different age groups of the populations, showing those with significant differences.

			GW/BW		Maturation Score	
Sex	Population	Age	Whole year	Months with variable age structure	Whole year	Months with variable age structure
M	Kelvin	0	.0082 \pm .0010	.0131 \pm .0002	45.3 \pm 5.9	20.9 \pm 10.4
M		1	.0079 \pm .0006	.0108 \pm .0026	63.6 \pm 5.6	16.7 \pm 16
M	Luggie	0	.0116 \pm .0009	.0132 \pm .0021	41.8 \pm 5.7	33.8 \pm 10.6
M		1	.0104 \pm .0011	.0130 \pm .0026	45.3 \pm 8.9	78.2 \pm 22.6
M	Aurs Burn	0	.0120 \pm .0017	.0088 \pm .0007	57 \pm 5.5	22.1 \pm 7.8
M		1	.0094 \pm .0021	.0139 \pm .0068	64 \pm 5.9	71.3 \pm 10.8
M = MALES; * = P < .05; ** = P < .01						

Table 5.2a Sample sizes used in Table 5.2

Whole year sample size (N)

Kelvin females: Age 0 (N = 80); Age 1 (N = 83); Age 2 (N = 4).

Luggie females: Age 0 (N = 67); Age 1 (N = 31)

Aurs Burn females: Age 0 (N = 76); Age 1 (N = 68); Age 2 (N = 3)

Months with variable age structure, sample size (N)

Kelvin females: Age 0 (N = 23); Age 1 (N = 9)

Luggie females: Age 0 (N = 11); Age 1 (N = 3)

Aurs Burn females: Age 0 (N = 9); Age 1 (N = 22)

Table 5.3b Sample sizes used in Table 5.3

Whole year sample size (N)

Kelvin males: Age 0 (N = 63); Age 1 (N = 58)

Luggie males: Age 0 (N = 61); Age 1 (N = 28)

Aurs Burn males: Age 0 (N = 74); Age 1 (N = 48)

Months with variable age structure, sample size (N)

Kelvin males: Age 0 (N = 15); Age 1 (N = 6)

Luggie males: Age 0 (N = 16); Age 1 (N = 5)

Aurs Burn males: Age 0 (N = 23); Age 1 (N = 12)

5.3.4 Association between body features and gonadal development

The analysis in this section was carried out to identify possible relationships between somatic growth and reproductive development. In the tables, all significant results are positive except where a negative relationship is indicated.

In the samples from the Rivers Kelvin and Luggie, some fish of as much as 2.7cm had undifferentiated gonads. In none of the fish from the Aurs Burn was this the case, even though plenty of small fish were sampled. On the other hand, there were cases from all three populations in which smaller fish (1.9cm) could be sexed. These observations suggest that gonads mature early in Aurs Burn fish and that within a given population, there is considerable individual variability in the rate at which gonads mature.

Length versus gw/bw ratio and maturation score

Table 5.4 shows a summary of results obtained by regressing gw/bw ratio and maturation score on length. Gw/bw ratio was positively related to length in 0 year old females of all three populations but not in older females. Similar results were also obtained for maturation score though the positive relationship persisted in one year old females of the River Luggie.

For males, there was no relationship between gw/bw ratio and length for any of the 3 populations. In contrast, maturation score was positively related to length in zero year old males of all 3 populations and also for one year old males of the River Luggie and Aurs Burn.

Table 5.4 Summary of results obtained from regressing gonad weight/body weight ratio and maturation score (ms) on length.

Sex	IV(x)	Population	Age	Gw/bw(p value)	MS(p value)
F	Length	Kelvin	0	**	**
"	"	"	1	NS	NS
"	"	"	2	NS	NS
F	Length	Luggie	0	**	**
"	"	"	1	NS	*
F	Length	Aurs Burn	0	**	**
"	"	"	1	NS	NS
"	"	"	2	NS	NS
M	Length	Kelvin	0	NS	**
"	"	"	1	NS	NS
M	Length	Luggie	0	NS	**
"	"	"	1	NS	**
M	Length	Aurs Burn	0	NS	**
"	"	"	1	NS	**

IV = Independent variable; Gw/bw = Gonadweight/bodyweight; MS = Maturation score; M = Males; F = Females; * = $p < .05$; ** = $p < .01$; NS = Not significant.

Condition factor versus gw/bw ratio and maturation score

Table 5.5 shows results obtained from regressing gw/bw ratio and maturation score on condition factor. There was a positive relationship between gw/bw ratio and condition factor for one year old females from the Kelvin and Aurs Burn but not for any other age categories. Only one year old females from the Kelvin had a positive relationship between condition factor and maturation score.

In one year old males from the Kelvin and Aurs Burn, gw/bw ratio was negatively related to condition factor. There was no relationship between gw/bw ratio and condition factor for any other male category. 0 year old males from the Aurs Burn had a negative association between maturation score and condition factor while 0 year old Luggie and Kelvin males had a positive relationship.

Table 5.5 Summary of results obtained from regressing gonad weight/body weight ratio and maturation score on condition factor.

Sex	IV(x)	Population	Age	Gw/Bw(p value)	MS(p value)
F	CF	Kelvin	0	NS	NS
"	"	"	1	*	*
"	"	"	2	NS	NS
F	CF	Luggie	0	NS	NS
F	"	"	1	NS	NS
F	CF	Aurs Burn	0	NS	NS
"	"	"	1	**	NS
"	"	"	2	NS	NS
M	CF	Kelvin	0	NS	*
"	"	"	1	*(negative)	NS
M	CF	Luggie	0	NS	*
"	"	"	1	NS	NS
M CF	Aurs Burn	0	NS	** (negative)	
"	"	"	1	*(negative)	NS

IV = Independent variable; Gw/Bw = gonad weight /body weight; MS = Maturation score; F = Females; M = Males; Cf = Condition factor; * = $p < .05$; ** = $p < .01$; NS = Not significant.

5.3.5 Relationship between stomach fullness and morphological development

Table 5.6 summarizes the results from this test. Testing all females and all males, there was a significant positive relationship between stomach fullness and condition factor. When different populations were taken separately however, for Luggie males and females there was no relationship between percentage stomach fullness and condition factor. Both males and females from the Aurs Burn had a significant positive relationship between stomach fullness and condition factor while for Kelvin males and females , the relationship was negative.

All the fish from the 3 populations tested together showed a positive relationship between stomach fullness and both gonad weight and gw/bw ratio. Similar results were obtained testing all females together but not all males. Taking males and females of the 3 populations separately however, the significant positive relationship between percentage stomach fullness and gonad weight, gw/bw ratio only persisted for the Aurs Burn fish.

There was a significant positive relationship between percentage stomach fullness and maturation score taking all females together but not with all males. Taking populations separately, Kelvin males and Aurs Burn females had a positive relationship between percentage stomach fullness and maturation score but for Luggie males, the relationship was negative.

Any possible association between percentage stomach fullness and condition factor could be an artefact of the fact that the weight of stomach content is included in the overall somatic body weight used in the calculation of condition factor. To see if this was the case, the analysis was repeated for those groups that had a significant relationship between condition factor and percentage stomach fullness. The condition factor was recalculated after the weight of stomach contents had been subtracted from the body weight, but significant levels still persisted.

Table 5.6 Summary of results showing the relationship between percentage stomach fullness and different morphological features. All significant association are positive unless indicated otherwise.

Population	Sex	Length	Weight	CF	GW	GW/BW	MS
Whole fish	MF	NS	SIG	SIG	SIG	SIG	-
"	F	NS	NS	SIG	SIG	SIG	SIG
"	M	NS	NS	SIG	NS	NS	NS
KELVIN	F	NS	NS	SIG(-ve)	NS	NS	NS
"	M	NS	NS	SIG(-ve)	NS	NS	NS
LUGGIE	F	NS	NS	NS	NS	NS	NS
"	M	NS	NS	NS	NS	NS	SIG(-ve)
Aurs Burn	F	NS	NS	SIG	SIG	SIG	SIG
"	M	NS	NS	SIG	SIG	SIG	NS

NS=Not significant, SIG=Significant, CF=Condition factor, GW=Gonad weight, Gw/bw=Gonad weight/boby weight, MS=Maturation score.

5.4 DISCUSSION

The annual reproductive cycle of the three spined sticklebacks described in this chapter generally agrees with earlier investigations (Craig - Bennett 1931; Tromp - Blom 1959; Borg 1982; Wootton et al 1976). Gonad weight in females was highest just before and during the breeding season, though this was less marked for River Luggie females. Both Wootton et al (1978) and Borg and Veen (1982) observed the same for their study populations. Though the different stages of the development of the eggs were present throughout the year, the more developed stages were dominant as the breeding season approached. Borg and Van Veen (1982), though they looked at ovaries at relatively fewer times in the year as well as using a different criteria for grouping the oocytes, found similar results.

Both Borg (1982) and Wootton (1984) report that the testes reach their maximum relative size in the autumn and then a decline in relative size follows. Borg also showed that spermatogenesis is completed in January after which the testes are largely occupied by spermatozoa and contain only few cells of other spermatogenic stages. Craig - Bennett (cited above) however still found active spermatogenesis in spring, but both authors agree that spermatogenesis is largely quiescent during the breeding season and commences at the end of it (July to August). In the present study, as in previous ones, relative testis weight varied little across the year compared to relative ovary weight. Relative gonad weight was however high for males of the three study populations in the autumn between September and November; Spermatogenesis was not however

completed as early as reported in Borg's study (cited above). Only Aurs Burn males had high levels of spermatozoa in December. Spermatozoa could be observed by January for the Luggie and February for the Kelvin males but levels did not become high until April. So active spermatogenesis was still going on and the earlier stages could also still be observed during this period. This is to be expected in these 3 populations studied which have a protracted breeding season and have small fish (born the previous season) in April with still immature gonads.

The results analysed in the present chapter indicate that fish with well developed eggs and sperm are rare in samples collected later than June 1984. This makes the fact described in chapter 3 that young fish continue to be recruited into the population in August and September hard to understand. There are two possible explanations for this discrepancy, which are not mutually exclusive:

(a) 1984 (when the gonad analysis was carried out) was a short breeding season. Pilot studies showed that in 1983 (when the late recruited fish of this sample hatched) adult males and females with well developed gonads were found later in the season.

(b) During sampling, it became apparent that late breeding fish were nesting in deeper water where they were very difficult to catch; fish with well developed gonads were therefore under represented in the samples.

On the question of possible relationships between size and gonadal development, the present study showed that for females of all 3 populations, the largest fish had the best developed gonads. Wootton (1973; 1977) has shown that in the breeding season heavier females

also produced the greatest number of eggs per spawning. For males from all 3 populations, larger fish had higher maturation scores although size was not related to relative gonad weight. Borg (1982) did not relate size to gonad development in the males he studied and so the generality of these present results is not clear. Since late hatched fish are smaller than those hatched early in the season, this positive relationship between size and gonadal development means that late hatched fish are also less mature. It is indeed the case that in fish of less than 3cm caught in the period just prior to the breeding season, gonads consist almost entirely of early developmental stages.

On the subject of population differences, Kelvin females were slightly longer than Aurs Burn females while both were longer and heavier than Luggie females. Kelvin males weighed more than the males from the other two populations but were only significantly longer than the Luggie males. However, for neither sex were there population differences in gonad weight or gonad weight/body weight ratio. So differences in size in the 3 populations was not accompanied by differences in absolute or relative gonad weight. However, the Luggie fish which were the smallest, had the lowest maturation score for both sexes (though this was only significant for the males).

Can any of these differences in size and gonad development be related to body condition? Fish of both sexes from the Luggie (whose maturation score were low) had the highest condition factor while the Aurs Burn males and females (with high maturation score) had the lowest condition factor. Aurs Burn fish seem therefore to be

investing in growth and gonadal development at the expense of condition factor; while Luggie fish have poor growth and gonad development but good body condition. Wootton (1973; 1977) working on mature adult females showed that the cost of egg production had to be met by the food consumed and that whenever food was in short supply, resources were transferred from the somatic tissues to the ovaries. Egg production is such an important component of fitness that the high condition factor and low maturation score of the Luggie females are surprising. The winter flounder (Pseudopleuronectes americanus), studied by Tyler and Dunn (1976) also seemed to be adopting a strategy of maintaining body condition at the expense of egg production when food was in short supply. They gave the flounders different food levels and concluded that they sacrifice egg production and maintain body size so that come a good year, the body size will be large enough to support a large ovary. Luggie females however do not generally have this opportunity of a second breeding episode in the subsequent years.

In the present study for both sexes, condition factor started to rise early in the year and the fish reached their best body condition as they approached the breeding season. Body condition then deteriorated, but this probably reflects recruitment of young fish into the population rather than poor condition of post breeding adults. Kelvin males were an exception with high condition factor all year except in September. Wootton et al (1978) found similar results for females in two stickleback populations (Llyn Frongoch and River Rheidol) and Pennycuik (1971) studying a population of sticklebacks in S. W. England found that condition factor was also high in spring and a drop occurred in May, which she ascribed to the

energetic costs of spawning.

Regression analysis within populations showed that females (in River Kelvin and the Aurs Burn) in good condition had well developed gonads. For older Kelvin and Aurs Burn males however, relative gonad weight was negatively related to condition factor, so gonadal development might be occurring at the expense of body condition. These one year old males are for the most part breeding males, so this negative relationship could also reflect the fact that at this time of the year males with developed gonads breed. Since breeding behaviour is energetically expensive and interferes with feeding, fish use their food reserves which in turn decreases their condition factor.

How do condition factor and growth relate to food availability? Percentage stomach fullness is not an ideal measure of food availability and at best reflects present food levels rather than those prevailing at the time growth was going on. However there was not enough time to do an ecological survey, and it was hoped that any major differences between populations in food levels would be picked up by this measure of percentage stomach fullness. Using this measure to analyse differences between individuals within a population is even more dubious since any relationship between present stomach fullness and gonad development (which reflects growth in the past) would depend on there being consistent individual differences in foraging efficiency. With these provisos in mind, it seemed worthwhile to look for a relationship between food intake, gonadal development and body condition.

There was no relationship between stomach fullness and either length or weight in any of the 3 populations. In fish from the Aurs Burn (which had low stomach fullness) both males and females had a positive relationship between percentage stomach fullness and condition factor. In the same population, fish with higher percentage stomach fullness also had higher gonad weight as well as higher gonad weight/body weight ratio. Fish from the Aurs Burn seem to be up against a food constraint, so that both condition factor and gonadal development are influenced by food supply.

Wootton's studies on female sticklebacks (1973, 1977) have clearly shown that females with experimentally higher rations during the breeding season had better developed gonads and produced a greater number of eggs and that the development of the ovary was always given priority to somatic growth. Although Wootton's study is conclusive, it is not clear to what extent this sort of constraint is important in the wild. The present results (using data collected over the whole year) shows low food intake to be a constraint only for the one population for which food seemed to be in short supply.

In the Luggie and Kelvin fish (both of which have a good food supply), percent stomach fullness was not related to any of the body parameters except for a few odd results for which no explanations was found (such as negative relationship between condition factor and stomach fullness in both sexes of the Kelvin fish).

There was no evidence of somatic growth occurring at the expense of gonadal development in the females of the 3 populations. Females in good condition had well developed gonads. A negative association did

exist for older Kelvin and Aurs Burn males; relative gonad weight was negatively related to condition factor.

This chapter has therefore shown that females have more mature stages of the ova during the breeding season and for the males, high levels of spermatozoa appeared at different times of the year, being earliest for males from the Aurs Burn. Females have a positive relationship between age, size and gonadal development but this was not the case for males. Older males however have a negative relationship between gonadal development and body condition. Only the Aurs Burn fish (which had the least food intake) had a significant positive relationship between percentage stomach fullness and body condition; these fish seemed to be investing on gonadal development at the expense of body growth.

CHAPTER 6

SEASONAL INFLUENCE ON RISK-TAKING DURING REPRODUCTION IN STICKLEBACKS

6.1 INTRODUCTION

The assumption underlying much modern research into the adaptive significance of behaviour is that, through the workings of natural selection, animals have combinations of traits which maximize their life time production of surviving young. Among the characters which have been investigated in this framework is the investment (including behavioural) that animals make in their offspring.

In his classic paper Trivers (1972) defined parental investment as "any investment made by an animal in one of its offspring which increases the latter's chances of survival at the cost of the parent's ability to invest in other offspring" and predicted that parental behaviour towards a current brood will be determined by the amount of previous parental investment in that brood. This position has been criticized by Dawkins and Carlisle (1976) who point out that expected future payoffs are more critical than past investment.

Thus Robertson and Bierman in 1979 showed that parental investment is determined by expected future benefits minus expected cost from the present breeding episode, rather than by the accumulation of past parental investment. They manipulated clutch sizes of nesting Redwinged blackbirds (Agelaius phoeniceus) to obtain a group of females with similar cumulative past investment but different expected future benefits. Measurement was then made of aggression directed to a dummy predator at the nest. They found that females with larger clutches were significantly more aggressive to the predator at the nest than those with smaller clutches, even though past investment was the same.

Studies of the selective forces influencing risk taking by parents in defence of their young suggest that at least two life history parameters are important, the value of ^{the} brood and the life expectancy of the parent. Forester (1983), studying the duration of brooding in the mountain dusky salamander (Desmognathus ochrophaeus) and its influence on aggression, observed that females attending eggs near to hatching showed more aggression to a conspecific male than those attending recently laid eggs. Curio et al (1984), also found that in great tits (Parus major) defending a brood, the strength of response and associated risk increase with advancing time in the breeding season. In these two studies, the value of the brood was the important factor. In another study (Pugesek 1983), older California Gulls (Larus californicus) were found to defend their brood more rigourously than younger ones. Here life expectancy of the parent was the main influential factor; low expected future payoffs from later broods leading to high investment.

In sticklebacks parental investment by males takes the form of building nests, fanning, defending their territory and the young it contains. All of these are conspicuous activities and therefore likely to increase predation risk. In addition sticklebacks defending eggs and young are more aggressive towards an intruder and bolder in the presence of a predator (Huntingford 1976a). Parental behaviour is therefore risky and the costs incurred probably increase later in the parental cycle. That levels of aggression are adapted to predation risk, among other things, is suggested by an observation (Huntingford 1982) that males from highly predated sites tend on average to be less aggressive to other males (though other factors are important, Giles and Huntingford, 1984). In foraging

theory it is becoming increasingly clear that the presence or absence of predators influences the optimal solution; a classic example is the study on sticklebacks by Milinski (1979). The present study looks at the impact of the presence of predators on behaviour in a quite different context, namely territorial defense, in an attempt to identify the factors that determine risk taking.

Pressley (1981) investigated the risks taken by parental male sticklebacks when caring for broods of different reproductive value in the field. The intensity of attack shown by parental males towards a dummy sculpin (a recognised predator of sticklebacks) was higher for larger broods and for males with older eggs in their nest, both of which are more valuable.

The previous chapters have shown that, in the Scottish populations of sticklebacks used in the present study, only offspring hatched earlier on in the breeding season (which lasts for about four months) are likely to reach maturity and thus to breed at the next breeding season at (at an age of 1 year). Offspring bred towards the end of the season are not usually mature at (at an age of 1 year) and their chances of surviving to breed in the subsequent year (at two plus) are slim. Broods hatched earlier on in the season may therefore be more valuable and this may be reflected in a higher degree of parental investment. On the other hand, this study has also shown that the potential for future breeding episodes in males from these populations is negligible. Theory suggests that this would be reflected in greater risk taking by late breeding males. This is obviously a complicating factor since it leads to two conflicting predictions. The present study was designed to clarify the position,

with the following aims in mind:

1. To find out whether breeding male sticklebacks adapt their reproductive behaviour to the presence of a predator.
2. If so, to see whether the behavioural changes are influenced by the presence of eggs in the nest .
3. To see whether the behavioural changes are influenced by the stage of the breeding season.

Studies were therefore made on how breeding male sticklebacks divide their time between nesting, territorial and self defense in the presence and absence of a predator. Tests were carried out on males with and without eggs in their nest and also at different times of the season to find out if time allocation is influenced by the value of the brood. Since age structures of the three study populations were very similar, and pilot experiments on the allocation of time to different activities by breeding male sticklebacks of the three populations showed no differences between populations, these behaviour studies were carried out on males from the River Kelvin only.

6.2 MATERIALS AND METHODS

Breeding male sticklebacks were collected from the River Kelvin every two weeks starting from the end of April till the beginning of August 1984 and brought back to the laboratory (the 1984 breeding season ended earlier than was observed in the previous year when breeding was still going on until September). Each breeding male was then placed in a tank divided into two compartments (sizes 70x35x38 cm

and 30x35x38 cm) by a transparent partition and an opaque partition was used to separate the predators at the times they were not needed during the test. The male was allowed to build a nest in the larger compartment and predators were placed in the smaller compartment when required during each test. Two identical sets of tests each of 15 minutes duration was carried out on each fish. The first, referred to as the empty nest test, was carried out three days after nest construction had been completed. After the empty nest test, the male was presented with a gravid female (similar sized females were chosen as there was need to keep the variation in number of eggs laid to a minimum), allowed to court her and to fertilize a single clutch of eggs in his nest. Three days later, the second of experiment (the egg test) was then carried out.

During each test, the male was presented with an intruder in form of another male conspecific in breeding condition. The intruding male was placed in a round glass tube and lowered into the tank 20cm away from the nest of the resident male. Two trout (measuring between six to ten inches, a size large enough to represent a potential threat) were used as predators and housed in the smaller compartment of the tank where they could be seen by sticklebacks when an opaque partition was lifted for specific periods during the experiment.

The 15 minute duration of the test was divided into three five minute blocks. The trout predators were only visible in the second block of five minutes and were absent in the others. The conspecific intruder was however present throughout the 15 minute period. The design of this experiment was so as to observe how a resident male reacts to a male intruder alone, how the presence of a predator

* Because significant variation between males in both aggressive and anti-predator behaviour has been reported for sticklebacks, in these experiments each male was used as his own control to compare responses with and without eggs. Randomising the sequence in which the two tests were performed (by carrying out some empty nest test on males tending a second nest) would introduce a new set of confounding factors. For this reason, all fish were tested first in the empty nest condition and then with eggs. This raises the possibility of habituation both to the male conspecific and to the trout predator and indeed many of the differences between males with and without eggs are compatible with this explanation. The following points argue against the habituation hypothesis:

- (1) The 3 days gap is probably enough to remove any effects of habituation.
- (2) The trout predator was continuously moving and so was not a constant stimulus.
- (3) The fish had marked response in the presence of the predator in the 2 sets of the experiments and the males situation was not exactly the same because he had eggs in his nest on the second occasion.

affects this and how he recovers after removal of the predator.

In order to identify any influence of the presence of a predator on defense against territorial intruders, parental care and self protection records were made of the following behaviour patterns:

- (1) Bites at the intruding male: These were lunges at the tube with actual contact made usually with the mouth open.
- (2) Time spent within a ten cm radius of the tube.
- (3) Time spent at nest.
- (4) Time spent hiding in weed.
- (5) Duration of dorsal spine raising.
- (6) Visits to the partition: this occurred only during the second block of five minutes when the trout predators were present.

After the two sets of experiments, the number of eggs in the males nest was counted to check any possible variation over the breeding season. Altogether 29 males were tested and classified as belonging to three different time periods in the breeding season namely May, June and July.

* See opposite

6.2.1 Data analysis

The data was analysed for differences in behaviour at different times during each test and for differences between egg and empty nest males as well as the effects of season. For frequently occurring behaviour patterns (bites at the intruding male, time spent around the tube containing it, visits to the partition and duration of spine

raising), difference between groups was tested by a one sample t-test at .05 level of significance. Difference between early and late breeders as well as the difference in the number of eggs in the males nest in the three time periods were analysed using a Mann Whitney-U test.

For behaviour patterns that occur rarely (time spent at nest, time spent hiding in weed and bites at partition) a presence or absence (binary response) test was used. For example, when comparing bites at partition in a nest and egg test, a two by two table was cast as follows:

I No. of fish not biting in either level	II No. of fish biting in nest but not in egg level
III No. of fish biting in egg level and not in nest	IV No. of fish biting in both levels

Binomial test was carried out on numbers in cell II versus cell III (Maclaren, pers.comm.).

6.3 RESULTS

In section 6.3.1 the behaviour patterns are discussed in order of frequency of observation, comparing fish with and without eggs. Seasonal effects are then considered in section 6.3.2.

6.3.1 The effects of presentation of a predator

Figures 6.1 to 6.4 show mean and standard errors for each behaviour in each minute of the test averaged over all males tested, taking egg and empty nest males separately. Tables 6.1 to 6.4 summarize the significant differences.

No. of bites at tube

For the empty nest males, the number of bites increased over the first few minutes of the test. There was a dramatic reduction in bite rate for both groups when the predator was introduced, and this was followed by a gradual increase although there was no significant effect of removing the predator.

Comparing fish with and without eggs, no warm up effect was observed in egg males at the beginning of the test but for the empty nest males, there was an initial increase in bites. The number of bites was consistently slightly higher for egg males (but only significantly so in minute one). Comparison of the equivalent three five minute blocks (that is, the period before the predator was presented, during its presence and after it has been removed) in the empty nest and egg test showed no significant differences.

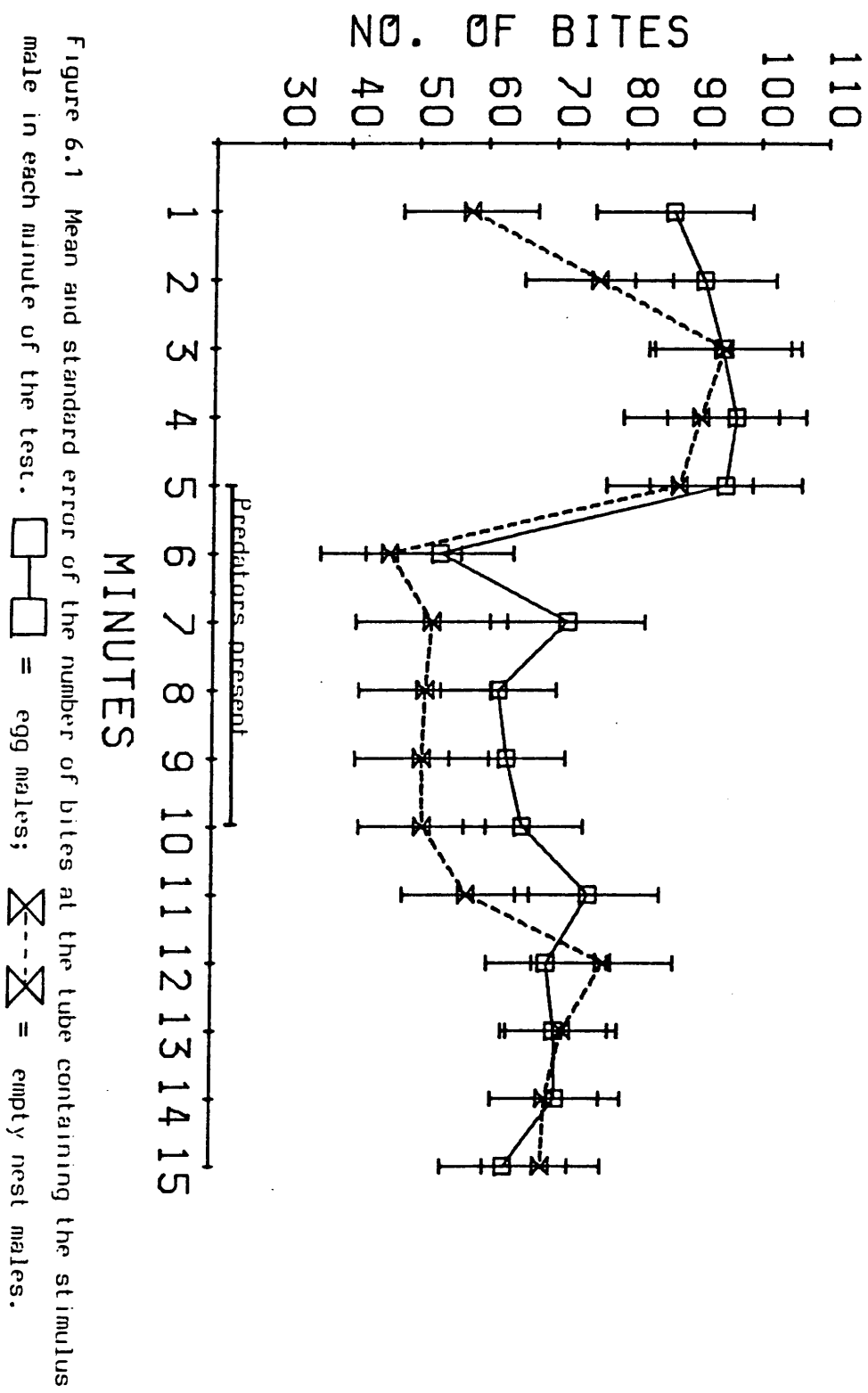


TABLE 6.1 Summary of minutes with significant differences for bites at tube.

	NESTTEST	EGG TEST
MINUTES 1 TO 5	1&2,1&3,1&4,1&5,2&3	NS
MINS. 5&6 (PREDATION EFFECT)	5&6	5&6
MINUTES 6 TO 10	NS	NS
MINS. 10&11 (REMOVAL OF PREDATOR)	NS	NS
MINUTES 11 TO 15	NS	NS
CORRESPONDING MINUTES IN NEST & EGG TEST	1	1
MINUTES 6 TO 15 (RECOVERY EFFECT)	6&15,8&13,7&14	NS
NS = NOT SIGNIFICANT MINS. = MINUTES		

Total time spent near tube

In each of the first five minutes of the test, the empty nest and egg males spent a large proportion of their time near the tube and on presentation of the predator, time spent at the tube dropped significantly. During the five minutes that the predator was present, levels remained depressed in both groups, gradually returning to normal by minute 11. Comparing fish with and without eggs, the average level of time spent near the tube in the first five minutes of the test was slightly higher for the egg fish but this was not significant. During the presentation of the predator, although levels remained depressed in both groups, egg fish spent significantly more time at the tube than the empty nest fish. Comparing the five minute blocks in the two groups showed a significant difference for minutes six to ten only.

Total time at partition

Stickleback males only came to the partition during the five minute period when the predator was present. In both groups, time at partition decreased during the test. While at partition, fish occasionally bit at the trout predator through the perspex. Comparing egg and empty nest males, empty nest males spent significantly more time at the partition (both minute to minute comparison as well as the five minutes pooled together) than the egg males, as well as having a slightly higher frequency of biting though this was not significant.

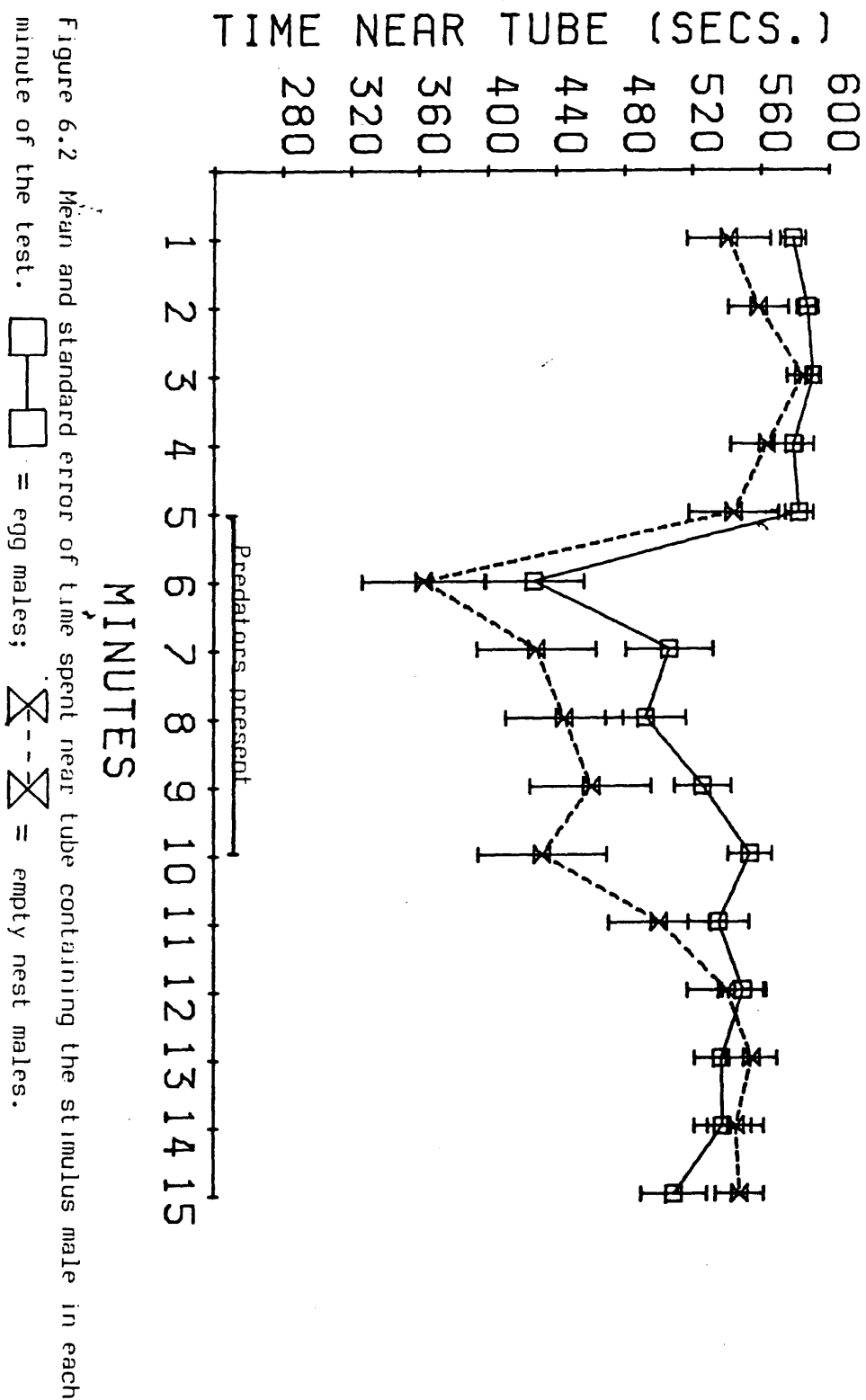


TABLE 6.2 Summary of minutes with significant differences for
time near tube.

	NEST TEST	EGG TEST
MINUTES 1-5	NS	NS
MINS. 5&6 (Predation Effect)	5&6	5&6
MINUTES 6-10	6&8,6&9	6&7,6&8,6&9, 6&10,8&10
MINS. 10&11 (Removal of predators)	10&11	NS
MINUTES 11-15	NS	NS
Comparing corresponding minutes in nest & egg test	10	10
NS = NOT SIGNIFICANT MINS. = MINUTES		

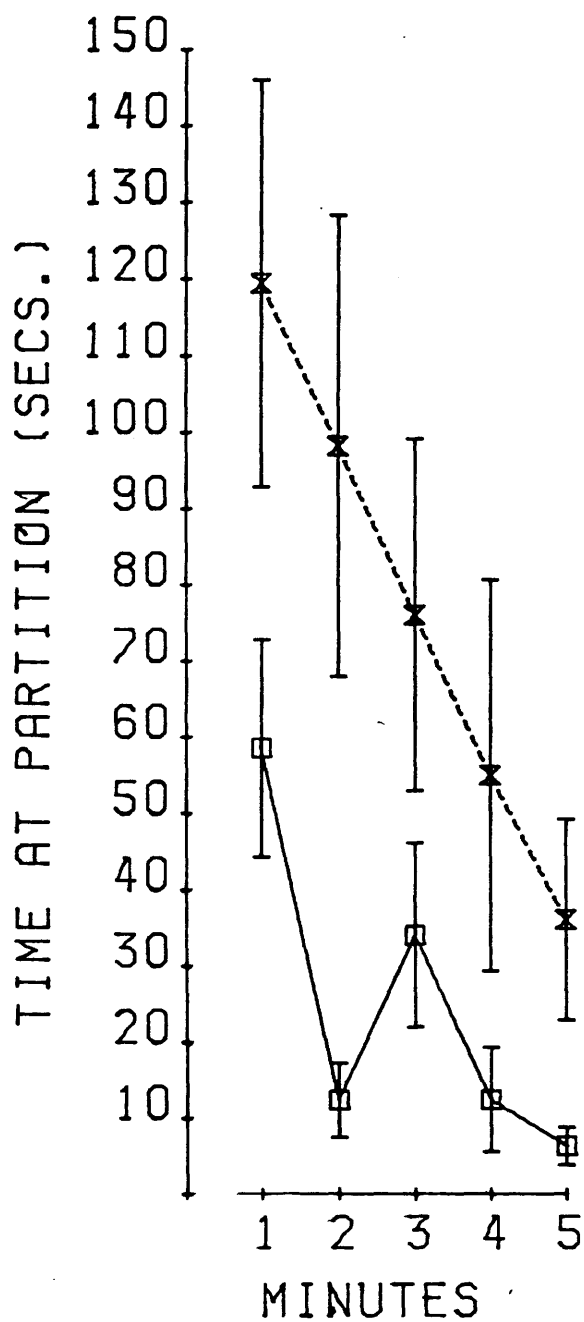


Figure 6.3 Mean and standard error of time spent at partition in the five minutes of test when the predators are present.

□—□ = egg males; X--X = empty nest males.

TABLE 6.3 Summary of minutes with significant differences for time at partition.

	NEST TEST	EGG TEST
MINUTES 6-10	6&9,6&10,7&10	6&7,6&9,6&10
COMPARING CORRESPONDING		
MINUTES IN NEST & EGG TEST	6,7,10	1,2,5

Duration of dorsal spines

The time spent with dorsal spines raised decreased over the first five minutes of the test in both groups, while presentation of the predator caused a significant increase. Over the subsequent ten minute period, spine raising gradually declined to pre-presentation levels in both groups. Comparing empty nest fish and egg fish, the empty nest fish raised their spines significantly more throughout the test. Each of the three five minute blocks in the two groups compared with their equivalent were significantly different.

Time near nest

Table 6.5 shows two by two tables cast for the binomial test as was previously explained. Tests were carried out on numbers in cell 11 versus cell 111. Throughout the fifteen minutes of the test, the egg fish visited their nests more but this was only significantly different from the empty nest fish in minutes 11-15.

Time spent hiding in weed

This behaviour did not occur in the first five minutes of the test when the predators were absent but subsequently the empty nest fish did more hiding; this was significant during the presence of the predators in minutes 6 to 10.

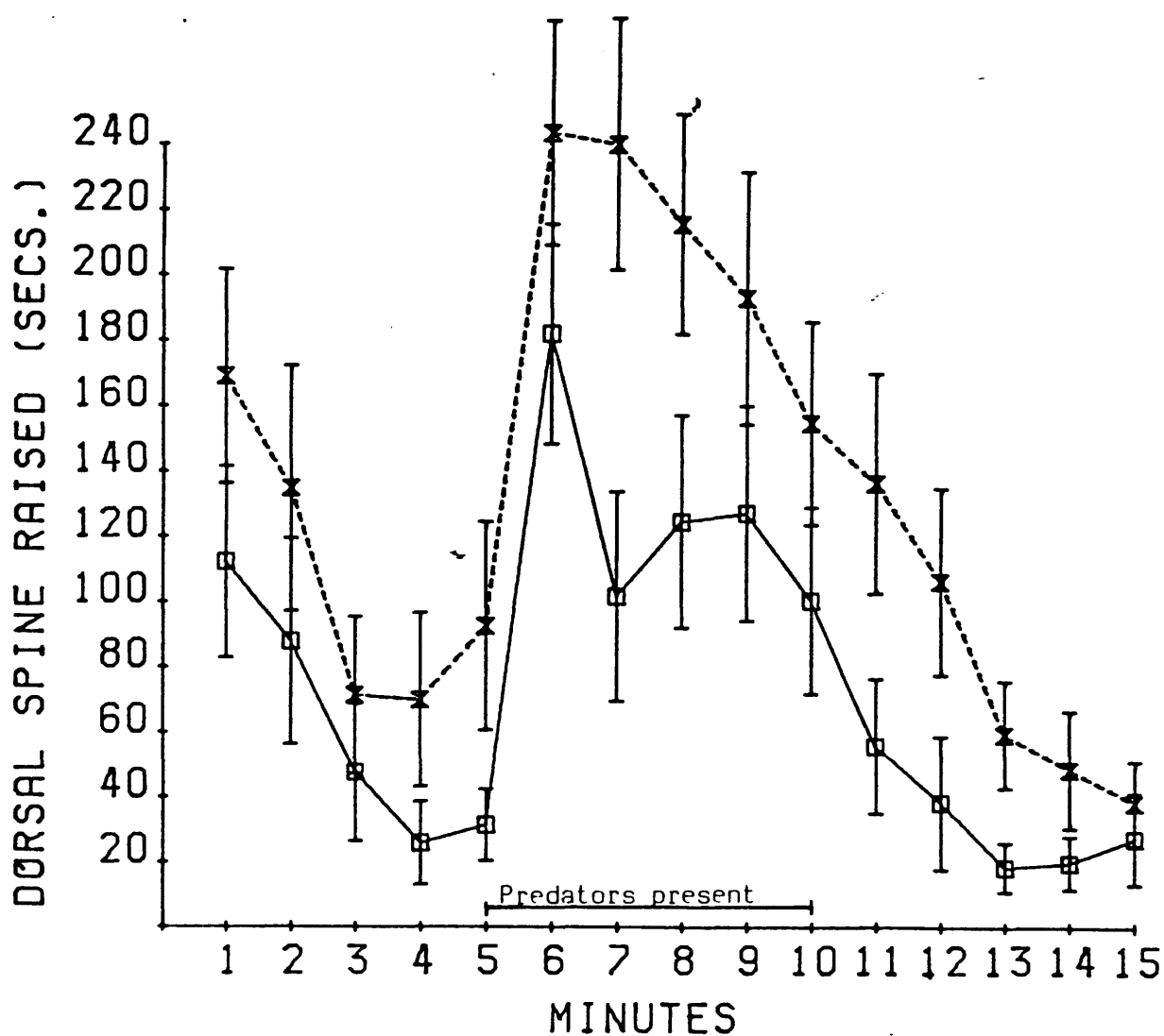


Figure 6.4 Mean and standard error of duration of dorsal spines raised in each minute of test. \square — \square = egg males; \times — \times = empty nest males.

TABLE 6.4 Summary of minutes with significant differences for duration of dorsal spine raising.

	NEST TEST	EGG TEST
MINUTES 1-5	1&3,1&4,2&3	1&4,1&3,1&5,2&4
MINS. 5&6 (PREDATION EFFECT)	5&6	5&6
MINUTES 6-10	NS	6&7,6&10
MINS. 10&11 (REMOVAL OF PREDATORS)	NS	NS
MINUTES 11-15	11&13,11&14,11&15 12&13,12&14,12&15	11&13,11&14
COMPARING CORRESPONDING		
MINUTES IN NEST & EGG TEST	4 5 7 8 10 11 12 13	4 5 7 8 10 11 12 13
NS = NOT SIGNIFICANT MINS. = MINUTE		

Table 6.5. A presence and absence table showing when only the egg males (cell 111) or only the empty nest males went to the nest (cell 11) as well as when both went (cell 1V) or neither went (cell 1).

		-	ENT	+
ET	-	1	11	
		21	2	
		111	1V	
	+	3	3	
Minutes 1 to 5				
		-	ENT	+
ET	-	1	11	
		13	5	
		111	1V	
	+	8	3	
Minutes 6 to 10				
		-	ENT	+
ET	-	1	11	
		8	4	
		111	1V	
	+	12	5	
Minutes 11 to 15				

ET = Egg test. ENT = Empty nest test.

Table 6.6. A presence and absence table showing number of times when only the egg males (cell 111) or only the empty nest males (cell 11) hid in the weed as well as when both went (cell 1V) or neither went (cell 1).

		-	ENT	+
ET	-	1	11	
		28	0	
	+	111	1V	
		0	0	
Minutes 1 to 5				

		-	ENT	+
ET	-	1	11	
		20	7	
	+	111	1V	
		1	1	
Minutes 6 to 10				

		-	ENT	+
ET	-	1	11	
		26	3	
	+	111	1V	
		0	0	
Minutes 11 to 15				

ET = Egg test. ENT = Empty nest test.

6.3.2 The effect of breeding early and late in the season

There was no difference in the number of eggs found in the males nest over the three time periods compared in this experiment.

Bites at tube

No significant differences in bites were found in the two blocks of five minutes before and after the presentation of the predator. During the presentation of the predator in minutes six to ten, empty nest fish made significantly more bites at the intruding male in May than in June and July (Fig. 6.5). Egg males (Fig. 6.5) did not differ in the number of bites they directed to the intruder between months in minutes one to five and six to ten. In minutes 11 to 15 however, the number of bites in May was significantly higher than for July.

Time near tube

A month effect was only observed in minutes six to ten in both groups. Empty nest males (Fig. 6.6) spent significantly more time near the tube in May than in June and July. For egg males (Fig. 6.6), the score in May was significantly higher than for July.

Time at partition

A significant month effect was only found in empty nest males (Fig. 6.7) where the partition was visited significantly more in June and July than in May. The median frequency for egg males was 0 for all the 3 months.

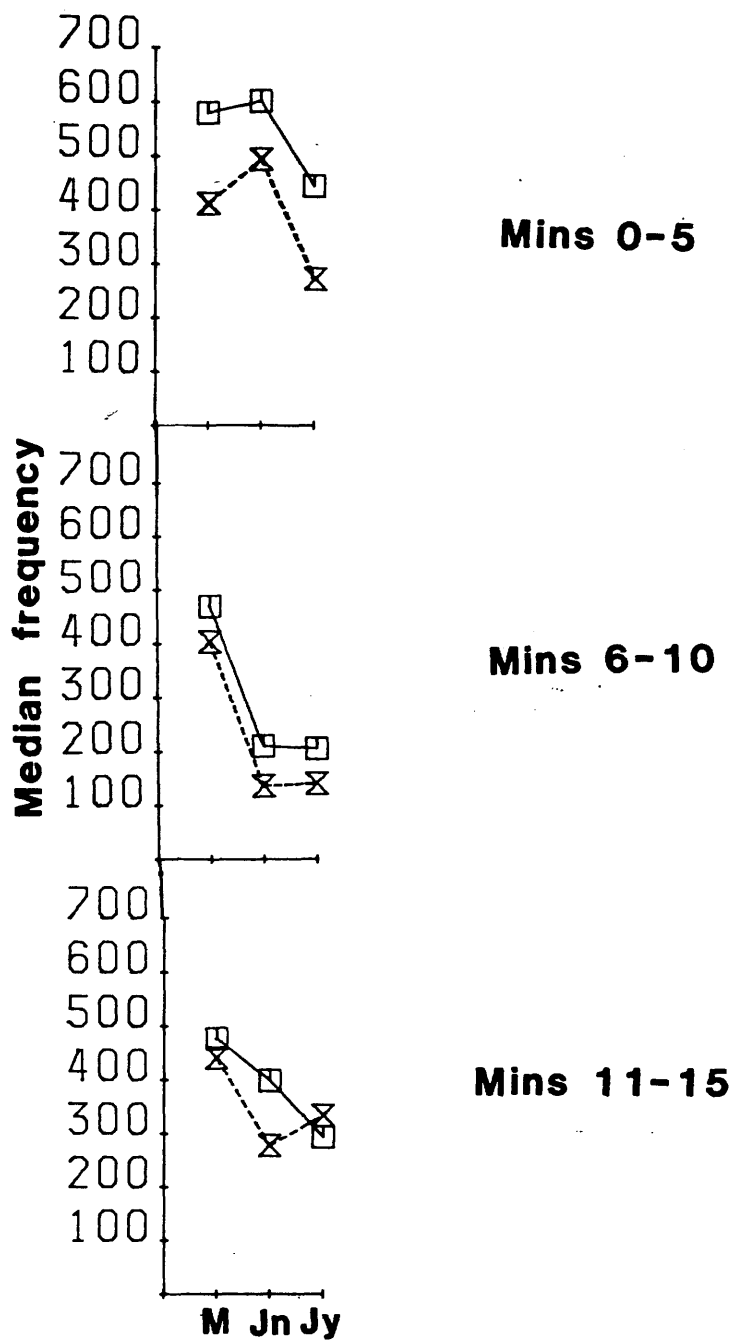


Figure 6.5 Mean number of bites at the tube containing the stimulus male in fish tested in May, June and July.

□—□ = egg males; X---X = empty nest males.

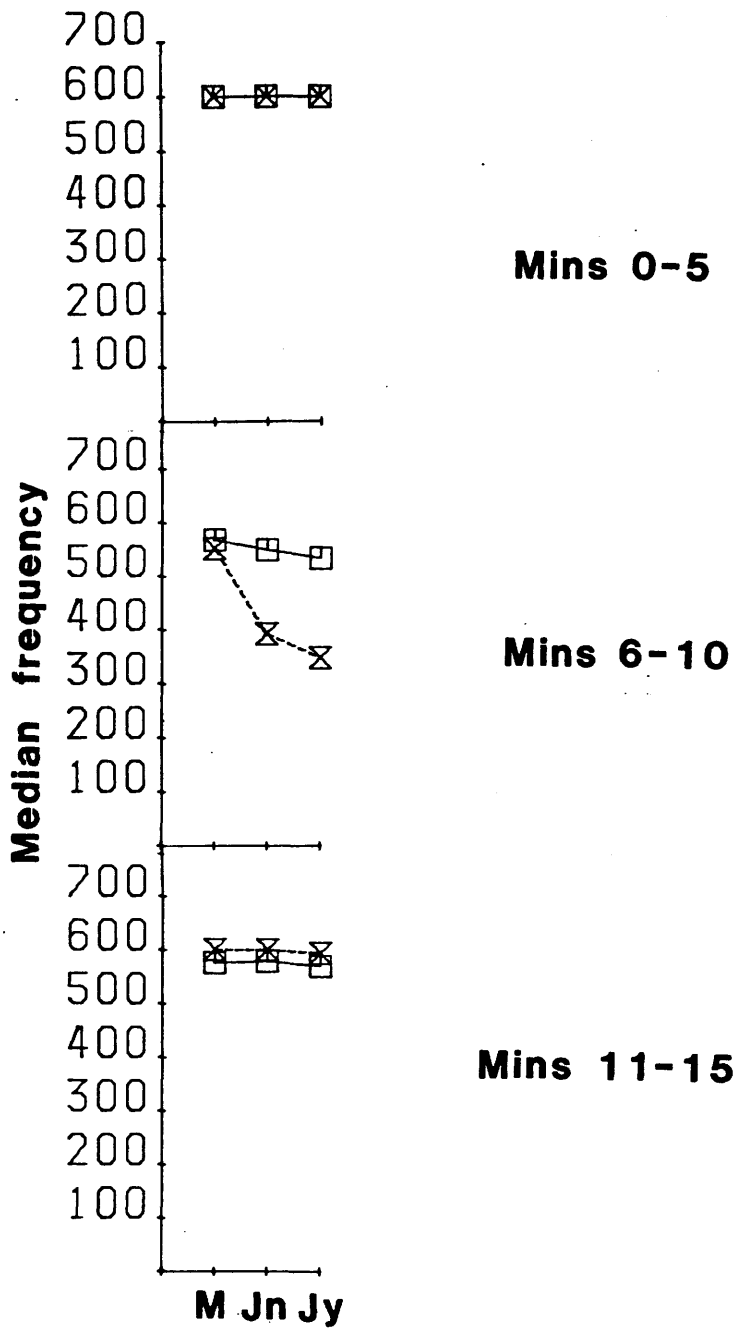


Figure 6.6 Mean time spent near the tube containing the stimulus male in fish tested in May, June and July.

□—□ = egg males; X---X = empty nest males.

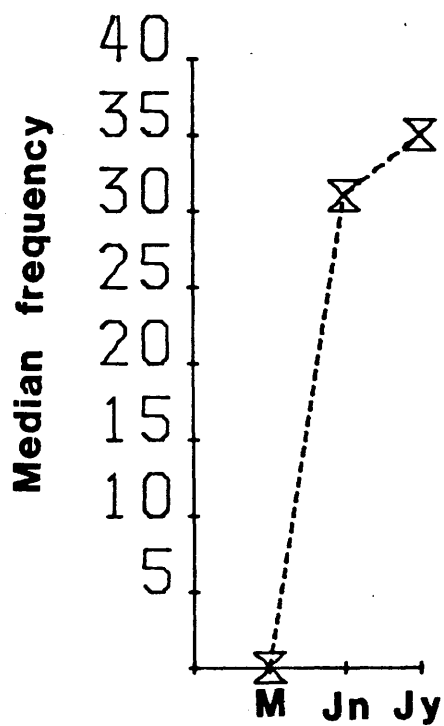


Figure 6.7 Mean time spent at partition in the five minutes of the test when the predators were present in fish tested in May, June and July. X--X = empty nest males.

6.4 DISCUSSION

The results confirm that breeding male sticklebacks do take risks to defend their young but that this behaviour is modified in response to the presence of the predator, so that a compromise is reached. The fish reduced their attack rate by almost half when the predator was presented but gradually recovered, so that removal of the predator *did* not have any effects.

Comparing egg males to empty nest males, the results show that the nature of the compromise changes, so that higher priority is given to nest care and defense of young and lower priority to anti-predator responses. This is reflected in higher levels of attack on an intruder by egg males and in lower level of hiding in weed and time at partition which seems to represent predator inspection. Similarly, as the breeding season advances the behaviour of males in both empty nest and egg test categories changes, with fewer risks being taken to defend young. This is more marked in the empty nest fish and is reflected in a decrease in the time spent attacking the intruder towards the end of the season particularly during the presence of the predator and an increase in time spent at partition.

The increased aggression towards intruders by egg males as well as the increased risks they take in the presence of the predator agrees with Huntingford (1976a and b). The fact that empty nest males spent more time at the partition appears to be directly opposite to Pressley's (1981) findings that the males with advanced eggs attack a predator more. One possible explanation is that the size of the male stickleback's territory contracts when they have eggs, therefore the

trout (though a risk) was outside their territory. Pressley's (cited above) dummy sculpin was lowered over the nest and so was clearly inside the territory of the fish. Another difference between this experiment and Pressley's is that, while presumably there were rival males around when he presented his predator, the fish did not have the immediate conflict between nest defense and predator avoidance that was imposed on the males in the present study. An alternative explanation is that in the present study, while at partition, sticklebacks are inspecting the predator so that this in fact represents an aspect of anti-predator behaviour. Investigation of predator is a commonly observed behaviour in a number of animals and has been described for sticklebacks (Reist 1983; Giles and Huntingford 1984) and minnows (Magurran and Pitcher 1986). So time at partition might reflect self defense and not the defense of brood.

Spine raising has a complicated motivational basis and this is different for dorsal and ventral spines. Dorsal spine raising seems to be controlled at least in part by fear. This is supported by Symons (1966) observations that biting is negatively correlated with the raising of dorsal spines. The fact that empty nest males raised their spines more in the present tests supports the picture of fish paying more attention to predators.

As mentioned in the introduction, two factors relating to life history theory change in River Kelvin sticklebacks as the breeding season progresses. Both the value of the young and the chances of the adult male breeding again decrease. The results discussed in this chapter suggests that the first of these, decreasing value of

brood has the stronger influence on risk taken by breeding male sticklebacks.

This is in contrast with two of the few comparable studies; Pugesek (1983) showed that parental California gulls take greater risks as they approach the end of their breeding life and Curio et al (1984) found that great-tits defending a brood of eggs take more risks as the breeding season progresses. So the result of the present study is suprising, given the almost non-existent chance that an adult male stickleback of this population will breed in two successive years. There are a number of possible explanations of this result, some of which are unlikely and others of which are possible.

Because the sticklebacks used in this study were collected from the wild just prior to testing, nothing is known about their past breeding experience. Theory suggests that future pay offs rather than past investment should determine behaviour during a current breeding episode. However the proximate cause of the differences between early and late breeding fish could be due to some physiological factor such as reduced energy reserves or generally poor health. The fact that late breeding fish are capable of maintaining high levels of aggression (there are no differences between early and late breeding fish before or after the predator is presented) rules out this possibility in any simple form. Moreover statistical analysis showed that Kelvin males caught in the wild over the breeding season did not differ in their body condition.

Since aggression puts sticklebacks at risk, selective predation on aggressive fish earlier in the breeding season may have left only

easily frightened fish later on in the breeding season. However, River Kelvin is not a heavily predated population so this explanation though plausible, is unlikely. It maybe that fish breeding later in the year are of poorer quality who have perhaps failed to establish territories earlier but again they do maintain high levels of nest defense in the absence of a predator. Thus there are a number of proximate mechanisms which could cause the decline in risk taking of the late breeding fish from the River Kelvin.

Further experiments could be carried out to verify some of these alternative explanations mentioned above. It would be interesting for instance to look at a population of sticklebacks with similar life history as the Kelvin fish but which in addition are heavily predated, to see if the drop off in parental care (late in the season) would be quicker. All the test fish could also be obtained at the beginning of the season and tested over the months so that their past breeding history is known.

Whatever the proximate mechanism responsible, there is still need to explain in functional terms why the male sticklebacks in this study do not appear to be adapting their behaviour to one critical aspect of their life history, namely their non-existent chances of a second breeding attempt.

As discussed in the introduction, stickleback of some populations live for two years or more. Males at the end of their first breeding season have other seasons ahead of them, but late hatched young would still be less valuable. Under such conditions, males would do better to increase their chances of survival even if this is at the expense

of their (not very valuable) late broods. Under these circumstances, the behaviour described in this chapter would be adaptive. If as seems likely the annual life cycle of Scottish sticklebacks is a derived condition, their apparently maladaptive behaviour maybe a hang over from ealier times.

The results discussed in this chapter have shown that breeding male sticklebacks take risks to defend their young and that the intensity of this attack is greater when they have a brood. Their breeding behaviour is influenced by the presence of a predator, and males with eggs take more risk to defend their brood compared to those with empty nest. Males that bred early in the season invested, more in their brood compared to those that bred late in the season.

CHAPTER 7
GENERAL DISCUSSION

This project describes a study of the age structure and various other life history parameters of sticklebacks (Gasterosteus aculeatus) from 3 Scottish populations. The populations are from Rivers Kelvin and Luggie and the Aurs Burn which are equivalent habitats (fairly fast flowing urban and suburban streams). These broadly similar sites were chosen to find out how variable or constant age structure is and how this relates to food intake. In this chapter the findings of the project are summarised and discussed in general terms. More detailed consideration is given in the appropriate sections of the thesis.

Length frequency distribution and otolith analysis were used to age the sticklebacks in this study and the age structure of the 3 populations have been characterized. Both methods used to age these fish showed that a great majority have a maximum life span of just over a year, although a few Kelvin and Aurs Burn females survived to 2 years (chapter 3). This same pattern of age structure was observed by Giles (1981) and Allen and Wootton (1982), but many other stickleback populations have a maximum life span of over 2 years (Jones and Hynes 1950); Greenbank and Nelson (1959).

It was noticed that a protracted breeding season (from late April to late August) occurs in all the populations. As a result, by the end of the breeding season in September, some fish (bred early in the season) had already attained a length of about 4 cm while some late-hatched fish were only just over 1cm long. The fish from these populations breed in their second summer when about a year old. However throughout the breeding season, we find as well as fully mature 1 year old fish, a category of 1 year old fish which are

comparatively small and not breeding, with gonads that are not fully developed (chapter 5). Various lines of evidence (including observation of late hatched fish kept in large holding tanks from hatching until breeding commenced) strongly suggest that these non breeding fish are offspring produced towards the end of the breeding season. Such non-breeding 1 year old fish are still found in July and August when the bulk of the breeding season is over. The present results show that their chances of surviving to breed in the subsequent year at age two plus are slim. In chapter 3, otolith analysis showed no male reproducing at age two plus and only very few females. It is not known if these two year old females are among the category that failed to reproduce at one year, though such an explanation has been proposed for two year old fish in Wales populations (Wootton 1976; Allen 1980).

Van Mullem (1967) in his study of *leius* stickleback populations in Geel Molen brook in the Netherlands concluded that they had two macro-breeding waves (with a period of about 2 months between them when no breeding was observed), the first in spring and the second in late summer. This resulted in a bimodal length distribution for fish bred in the same breeding season. We did not find 2 distinct waves of breeding, or a bimodal distribution of young, instead there was a tail of late breeders and a wide range of lengths in young fish.

The 3 populations had a similar pattern of length frequency distribution but on average Kelvin fish were longer and heavier compared to fish from the other two populations. The distribution of otolith types was also similar in the 3 populations but there was variation among individual fish as well as between populations.

Kelvin fish added the first transparent ring earlier than the other 2 populations. These population differences are in contrast to both Jones and Hynes (1950) and Greenbank and Nelson (1959) who reached the conclusion that the formation of the opaque and transparent rings may occur during different seasons in different species of fish but that the season of formation was constant for a given species.

Several studies have shown that food level is one of the major factors which affect variability in growth rate and life span in fish species. Studies on the food intake of sticklebacks are quite extensive (for example, Hynes 1950; Manzer 1976) but this is not usually related to age or gender of the fish, or to life history parameters such as body size and gonadal development. One of the aims of the present study was to monitor and compare the food intake (measured by percentage stomach fullness) of fish from the 3 study populations to try and fill in some of these gaps (chapter 4). Food intake was low in winter and this seems to agree with all the other studies (for example, Manzer 1976; Allen and Wootton 1982). Just before the breeding season, percentage stomach fullness was high but this dropped as breeding commenced fully, especially for males. Although Hynes (1950) noticed a similar decrease, both Manzer (1976) and Allen (1980) found that feeding levels were high throughout the breeding season.

There does not appear to be any simple relationship between gender age and food intake. Only in River Kelvin males did size have an effect on stomach fullness, with bigger males having relatively fuller stomachs. Population differences in stomach fullness did exist, with both sexes of the Kelvin and Luggie fish consuming more

food than those from the Aurs Burn.

While monitoring the food intake in the three populations, diet composition was looked at as a subsidiary aim and this showed *Asellus* and *Gammarus* to be the most important food items for the Kelvin and Luggie fish while chironomid larva was the commonest prey item in the Aurs Burn fish. Absence of large crustaceans from the diet of the Aurs Burn fish may be a reason for their low percentage stomach fullness. Maitland (1965) also found *Asellus* and *Gammarus* to be the major food items of sticklebacks in the River Endrick in Scotland. Manzer (1976) reached the conclusion that sticklebacks eat what is available to them in the wild, so that any difference in diet composition was unlikely as a result of changing diet preferences of the stickleback population.

Most studies on gamete development in sticklebacks have been descriptive rather than quantitative and all indicated variability particularly in the timing of maturation events. One of the broad aims of this project was to carry out detailed quantitative histological examination of the gonadal development of the fish and to relate systematic variation in population size and age structure to patterns of gonadal development.

The results discussed in chapter 5 show that the gonad weight of the females from all three populations was highest just before and during the breeding season, with the most developed stages of the ova predominating at this time. Both Wootton et al (1978) (studying the River Rheidol in Wales) and Borg and Veen (1982) studying Swedish populations obtained similar results. On the other hand, the testes

were most developed in the autumn, as earlier studies have shown but abundance of spermatozoa observed in the present study varied between the populations. The testes of the Aurs Burn males were filled with spermatozoa as early as December but such high levels did not occur in the other two populations until about April. Males from the Aurs Burn were also observed to have slightly red throat in the winter; a situation which normally occurs near the beginning of the breeding season in May. In Borg's (1982) study of Swedish populations, spermatogenesis was finished by January when the testis were filled mainly with spermatozoa. In contrast Craig-Bennett (1931) still observed active spermatogenesis in April in males of his leiurus populations in Cambridge, as was obtained in this study.

For the females of the 3 study populations, a clear relationship existed between age, size and gonadal development, with older and larger females having more developed gonads as reflected by both gw/bw ratio and maturation score. In males, size was not related to gw/bw ratio, but older and bigger sized males had higher maturation scores.

The gonadal development of the 3 populations was compared to see if this could be related to the previously reported differences in average body size. There were no population differences in gonad development for females or males; in general therefore differences in size could not be attributed to allocation of resources to gonadal development. However, Luggie fish, which were the smallest, had the lowest maturation score; these same Luggie fish had the highest condition factor. This is in contrast to the Aurs Burn fish which had the poorest body condition but the highest maturation score.

Both sexes of the Aurs Burn fish (which had the lowest food intake) seem therefore to be investing in growth and gonadal development at the expense of body condition while the Luggie fish are doing the reverse. The low body condition of the Aurs Burn fish agrees with Wootton (1973, 1977) who showed experimentally that for breeding females, when food is in short supply, development of the ovaries is given preference to somatic growth.

Regression analysis at individual level in each population in (chapter 5) shows that females in good body condition had well developed gonads, suggesting that gonadal development was not at the expense of body condition for females. For 1 year old males in the Kelvin and Aurs Burn however, well developed gonads are associated with poor body condition. Because of the age structure of the populations and the way age was assigned, adult males are 1 year old during the breeding season so this negative relationship maybe the result of the energetic costs of breeding.

The Aurs Burn fish (but not the Kelvin and Luggie) had a positive relationship between percentage stomach fullness and body condition. This may be because this population seems to be up against a feeding constraint. In this same population, fish with higher percentage stomach fullness had higher gonad weight and gw/bw ratio. Though percentage stomach fullness at best reflects present food levels rather than what was available when growth was going on (as discussed in chapter 5); this result suggests that when food levels are below a certain value, the amount of food a stickleback obtains may determine both body condition and relative gonad development. To this extent,

the present field study agrees with Wootton's experimental findings, that when food is in short supply, the female sticklebacks' (during the breeding season) resources are channelled to gonadal development at the expense of body growth (Wootton, 1973; 1977).

The level of food and predation in a population have been shown to be among the parameters that influence life history theory since they have an effect on adult and juvenile survivorship. Thus guppies life history patterns can depend on predation levels as Reznick and Endler (1982) have shown. They found that guppies in those Trinidad streams they studied mature and breed early in streams where predators of adults are common and breed later where they are rare. Food supply also influences allocation to growth and gonadal development. Hirshfield (1980) working on the Japanese medaka found experimentally that when food was in short supply, priority was given to gonadal development at the expense of somatic growth.

In general, the present study shows no such negative association between allocation of resources to growth and reproduction; rather female sticklebacks with best developed gonads tended to be the largest and in best condition. In the population that had the lowest food intake (Aurs Burn) however, they still maintained high gonadal growth but had comparatively low body condition. In older Kelvin and Aurs Burn males, a negative relationship exists between body condition and gw/bw ratio; this could represent the channelling of energy to gonadal development rather than to the body, but might also reflect the fact that males with well developed gonads breed and that this is expensive.

Among other things, this study showed that because of the protracted breeding season of these Scottish populations, late hatched sticklebacks unlike early hatched ones were not usually sufficiently mature to breed in their first full summer at 1 year. This suggests that broods hatched earlier in the season maybe of a higher reproductive value than those hatched late; the former have a much greater chance of producing offspring. If parental behaviour is tuned to the value of the brood (as suggested by life history theory), this difference in value of early and late broods might be reflected in a higher degree of parental investment by parents of the early broods. On the other hand, males from these same populations have negligible potential for future breeding episodes which leads to an opposite prediction.

Another aim of this thesis was to see whether breeding males maintain reproductive behaviour in the presence of a predator and to see whether this is affected by the presence of eggs and stage of breeding season. The results discussed in chapter 6 show that males do take risks to defend their young and that the presence of predators affects this response. Males having eggs take more risks in the presence of the predator. Aspects of the present result are in agreement with studies by Huntingford (1976a, b), Kynard (1978a) and Pressley (1981).

Kynard (1978a, b) in his detailed studies of parental behaviour of breeding male sticklebacks in lake Wapato (Washington) showed that males that spawned early in the breeding season had superior reproductive success compared to those that bred late (i.e about late August) which were also far less successful in rearing their eggs

* The complex models of life history theory outlined in chapter 1 investigate the way selective forces act on life history parameters but it is very difficult to obtain empirical data to test their predictions. One of the clearest predictions of life history models and one in which empirical data are available (Reznick and Endler 1982) is the role of predators and their influence on adult and juvenile mortality. One initial aim of this project was to compare life history parameters in predated and unpredated populations but this was not possible because of practical constraints.

One other prediction of life history theory is that trade-offs occur between somatic body tissue and in reproductive processes and empirical data have been collected to support this prediction (for example, Hirshfield 1980). The results of experiments described in the present thesis give little indication that gonadal development occurs at the expense of body condition in sticklebacks except for the fact that one year old males of the River Kelvin and the Aurs Burn may loose condition as a result of breeding adults.

Sibly and Calow's model (see table 1.1) predict trade-offs between parent and offspring survival. Behaviour studies in this project show that breeding males do indeed compromise their own chances of survival to enhance the survival of their brood, and that this is finely tuned to the value of the brood.

through to fry. No functional explanations were given for these observations. The lake Wapato sticklebacks have similar life history to the population of sticklebacks studied presently; about 98% of the adults die after the breeding season (Hagen and Gilbertson 1972). It is a possibility that the value of the brood towards the end of the breeding season (after July) was playing an important part in this observed parental behaviour of the late breeding males.

As has been indicated, life history parameters which might possibly influence parental behaviour of the male stickleback are value of brood and expected future reproductive effort of the males. The results obtained in this study indicates that the former has the stronger influence on risk taken by males. This is surprising and one suggested explanation is that this is behaviour which is adapted to biannual populations which might have been the original state of these stickleback populations.

Some of the trade-offs discussed in the model by Sibly and Calow (1983) (discussed in chapter 1) apply to breeding male sticklebacks in the present study. Breeding males in this study took risks defending their young (more so at the early part of the breeding season), and therefore were increasing the survivorship of their offspring while decreasing their own. By investing resources to protect their young, males were delaying recruitment of subsequent broods while increasing the survivorship of their present brood.

* see opposite

The main contributions of this thesis can be summarized as follows:

- (1) A detailed characterization of population age structure of 3 Scottish populations has been carried out and it was found that these sticklebacks have an average life span of just over a year.
- (2) Food intake of sticklebacks from these populations was monitored and related to age and gender of the fish. No simple relationship was found between age, gender and food intake. However differences in feeding intensity have also been related to life history parameters such as size, gonadal development and body condition.
- (3) A quantitative examination of the annual gonadal cycle and relative gonad and body weight of the 3 populations was performed. Relationship between different sizes and age of fish with gonadal development has been established. Older and larger females had better developed gonads as shown by both gw/bw ratio and maturation score. Older males only had higher maturation score. Only the Aurs Burn fish (which had low percentage stomach fullness) had a positive relationship between food intake and both gonadal development and body condition.
- (4) This study has established that late hatched fish in these stickleback populations (with a protracted breeding season) did not breed in the next season (following hatching) as did those hatched earlier in the season. This difference in the value of early and late

broods has been shown to influence the risk taken by parental male sticklebacks when defending their young in the presence of a predator.

References

- Abdel-Malek, S.A. (1962). Feeding of young three spined sticklebacks (Gasterosteus aculeatus L.) in Kandalaksha Bay in the white sea. Probl. Ichthyol. 8: 74-80 .
- Ahsan N. S. and Hoar W. S. (1963). Some of the effects of gonadotropic hormones on the three spine sticklebacks, (Gasterosteus aculeatus). Can. J. Zool. 41: 1045-1053.
- Allen, J. R. M. (1980). The estimation of natural feeding rate of the three spined stickleback (Gasterosteus aculeatus L.) (Pisces). PhD Thesis, University of Wales.
- Allen, J. R. M. and Wootton, R. J. (1982). Age, growth and rate of food consumption in an upland population of the three spined stickleback, (Gasterosteus aculeatus L.). J. Fish Biol. 21: 95-105.
- Alm, G. (1949). Influence of heredity and environment on various forms of Trout. Rep. Inst. freshw. Res. Drottningholm. 29: 29-34.
- Assem, J. van den (1967). Territory in the three spined stickleback, (Gasterosteus aculeatus L.). An experimental study in intra-specific competition. Behaviour suppl. 16: 1-164.
- Baggerman, B. (1957). An experimental study on the timing of breeding and migration in the three spined stickleback (Gasterosteus aculeatus L.). Arch. neerl. Zool. 12: 105-317.
- Baggerman, B. (1972). Photoperiodic responses in the sticklebacks and their control by a daily rhythm of photosensitivity. Gen. Comp. Endocrinol. Suppl. 3: 466-476.
- Balon, E. K. (1975). Terminology of intervals in fish development. J. fish. Res. Board Can. 32: 1663-1670.

- Baltz, D. M. (1984). Life history variation among female surfperches (Perciformes: Embiotocidae). Env. Biol. Fish. 10: 159-171.
- Baltz, D. M. and Moyle, P. B. (1982). Life history characteristics of tule perch (Hysterocarpus traski) populations in contrasting environments. Env. Biol. Fish. 7: 229-242.
- Benzie, V. L. (1965). Some aspects of anti - predator responses of two species of stickleback. D. Phil. Thesis, University of Oxford.
- Bertin, L. (1925). Recherches bionomiques, biometriques et systematiques sur les epinoches (Gasterosteides). Ann. Inst. Oceanogr. Monaco 2: 1-102.
- Blouw, D. M. and Hagen, D. W. (1981). Ecology of the four spine stickleback (Apeltes quadracus), with respect to a polymorphism for dorsal spine number. Can J. Zool. 59: 1677-1692.
- Bock, F. (1928). Die hypophyse des stichlings (Gasterosteus aculeatus L.) unter besonderer Beriicksichtigung der jahrescyklischen Veraderungen. Z. wiss. Zool. 131: 645-710.
- Borg, B. (1982a). Seasonal effects of photoperiod and temperature on spermatogenesis and male secondary sexual characters in the three spined sticklebacks, (Gasterosteus aculeatus L.) Can. J. Zool. 60: 3377-3386.
- Borg, B. and Veen, Th.van (1982). Seasonal effects of photoperiod and temperature on the ovary of the three spined stickleback, (Gasterosteus aculeatus L.). Can. J. Zool. 60: 3387-3393.
- Bryant, E. (1971). Life history consequences of natural selection. Cole's result. Am. Nat. 105: 75-76.
- Charnov, E. L. and Schaffer, W. M. (1973). Life history consequences

- of natural selection: Cole's result revisited. *Amer. Natur.* 107: 791-793.
- Cole, L. A. (1954). The population consequences of life history phenomena. *Quart. Rev. Biol.* 29: 103-137.
- Cole, S. J. (1978). Studies on the energy budgets of two fresh water teleosts. MSc Thesis, University of Wales.
- Constantz, G. D. (1974). Reproductive effort in Poeciliopsis occidentalis (Poeciliidae). *Southwestern Nat.* 19: 47-52.
- Constantz, G. D. (1979). Life history patterns of a life bearing fish in contrasting environments. *Oecologia* 40: 189-201.
- Craig - Bennett, A. (1931). The reproductive cycle of the three spined stickleback, (Gasterosteus aculeatus L.). *Phil. Trans. Roy. Lond. B219*: 197-279.
- Curio, E.; Regelman, K. and Zimmermann, U. (1984). The defence of first and second broods by great tits (Parus major) Parents: A test of reproductive sociobiology. *Z. Tierpsychol.* 66: 101-127.
- Dawkins, R. and Carlisle, T. R. (1976). Parental investment, mate desertion and a fallacy. *Nature*, 262: 131-133.
- Forester, D. C. (1983). Duration of brooding period in the mountain duskey salamander (Desmognathus ordiophaeus) and its influence on aggression towards conspecifics. *Copeia* 4: 1098-1101.
- Gadgil, M. and Bossert, W. H. (1970). Life historical consequences of natural selection. *Am. Natur.* 104: 1-24.
- Gibbons, J. R. H. and Gee, J. H. (1972). Ecological segregation between long-nose and black-nose dace (Rhinichthys) in the Mink River, Manitoba. *J. fish. Res. Bd. Can.* 29: 1245-1252.
- Giles, N. (1981). Predation effects upon the behaviour and ecology

- of Scottish Gasterosteus aculeatus L. populations. Ph.D. Thesis, University of Glasgow.
- Giles, N. and Huntingford, F. A. (1984). Predation risk and inter-population variation in anti-predator behaviour in the three spined sticklebacks, Gasterosteus aculeatus L. Anim. Behav. 32: 264-275.
- Glebe, B. D. and Leggett, W. C. (1981). Latitudinal differences in energy allocation and use during the fresh water migrations of American shad (Alosa sapidissima) and their life history consequences. Can. J. fish. Aquat. Sci. 38: 806-820.
- Glen, G. L. and Ward, E. J. (1968). Wet weight as a method of measuring stomach contents of walleyes, (Stizostedion vitreum vitreum). J. fish. Res. Bd. Can. 25: 1505-1507.
- Gottfried, H. and Mullem, P. J. (1967). On the histology of the interstitium and the occurrence of steriods in the stickleback (Gasterosteus aculeatus L.) testis. Acta Endocrinol. 56: 1-15.
- Greenbank, J. and Nelson, P. R. (1959). Life history of the three spined stickleback (Gasterosteus aculeatus L.) in Karluk lake and Bare lake, Kodiak island, Alaska. U. S. fish wildlife Serv. fishery Bull. 153: 537-559.
- Gross, M. R. (1984). Sunfish, Salmon, and the evolution of alternative reproductive strategies and tactics in fish. In fish reproduction: Strategies and Tactics (G. W. Potts and R. J. Wootton, eds) pp. 55-75. Academic Press, London.
- Hagen, D. W. and Gilbertson, L. G. (1972). Geographic variation and environmental selection in Gasterosteus aculeatus L. in the pacific North West America. Evolution 26: 32-51.
- Hirshfield, M. F. (1980). An experimental analysis of reproductive

- effort and cost in the Japanese medaka (Oryzias latipes). Ecology 61: 282-292.
- Hirshfield, M. F. and Tinkle, D. W. (1975). Natural selection and the evolution of reproductive effort. Proc. Nat. Acad. Sci. U. S. A. 72: 2227-2231.
- Hoogland, R; Morris, D. and Tinbergen, N. (1957). The spines of sticklebacks (Gasterosteus and Pygosteus) as means of defence against predators. (Perca and Esox). Behaviour 10: 205-237.
- Horn, H. S. and Rubenstein, D. I. (1984). Behavioural adaptations and life history. In J. R. Krebs and N. B. Davies (eds), Behavioural Ecology: an Evolutionary Approach, 2nd ed, Blackwell, Oxford, 279-298.
- Huntingford, F. A. (1976a). A comparison of the reaction of sticklebacks in different reproductive conditions towards conspecifics and predators. Anim. Behav. 24: 694-697.
- Huntingford, F. A. (1976b). The relationship between anti-predator behaviour and aggression among conspecifics in the three spined stickleback, (Gasterosteus aculeatus). Anim. Behav. 24: 245-260.
- Huntingford, F. A. (1982). Do inter and intraspecific aggression vary in relation to predation pressure in sticklebacks? Anim. Behav. 30: 909-916.
- Hynes, H. B. N. (1950). The food of fresh water sticklebacks (Gasterosteus aculeatus and Pygosteus pungitius with a review of methods used in studies of food of fish. J. Anim. Ecol. 19: 36-58.
- V Hyslop, E. J. (1980). Stomach content analysis - a review of methods and their application. J. Fish Biol. 17: 411-429.
- Jensen, A. L. (1985). Relations among net reproductive rate and life

- history parameters for lake white fish (Coregonus clupeaformis). Can. J. fish. Aquat. Sci. 42: 164-168.
- Jones, J. W. and Hynes, H. B. N. (1950). The age and growth of Gasterosteus aculeatus, Pungitius pungitius and Spinachia vulgaris, as shown by their otoliths. J. Anim. Ecol. 19: 59-73.
- Kimball, D. C. and Helm, W. T. (1971). A method of estimating fish stomach capacity. Trans. Am. fish. Soc. 100: 572-575.
- Kimura, D. K.; Mandapat, R. R. and Oxford, S. L. (1979). Method, Validity and Variability in the age determination of yellowtail rockfish (Sebastes flavidus), using otoliths. J. fish. Res. Bd. Can. 36: 377-383.
- Kynard, B. E. (1978a). Breeding behaviour of a lacustrine population of three spine sticklebacks (Gasterosteus aculeatus L.). Behaviour 67: 178-207.
- Kynard, B. E. (1978b). Nest desertion of male (Gasterosteus aculeatus). Copeia, 702-703
- Lambert, T. C. and Ware, D. M. (1984). Reproductive strategies of demersal and pelagic spawning fish. Can. J. fish. Aquatic Sci. 41: 15565-1569.
- Le Cren, E. D. (1947). The determination of the age and growth of the perch (Perca fluviatilis) from the opercular bone. J. Anim. Ecol. 16: 188-204.
- Leggett, W. C. and Carscadden, J. E. (1978). Latitudinal variation in reproductive characteristics of American shad (Alosa sapidissima): evidence for population specific life history strategies in fish. J. fish. Res. Board Can. 35: 1469-1478.
- Magurran, A.E. and Pitcher, T.J. (1986). Provenance, shoal size and organisation of predator evasion behaviour in minnow shoals

(in press).

- Maitland, P. S. (1965). The feeding relationships of salmon, trout, minnows, stone loach and three spined sticklebacks in the River Endrick, Scotland. *J. Anim. Ecol.* 34: 109-133.
- Mann, R. H. K. (1971). The population, growth and production of fish in four small streams in southern England. *J. Anim. Ecol.* 40: 155-190.
- Mann, R. H. K. and Orr, D. R. O. (1969). A preliminary study of the feeding relationships of fish in a hard water and soft water stream in southern England. *J. fish. Biol.* 1: 31-44.
- Manzer, J. I. (1976). Distribution, food and feeding of the three spined stickleback, Gasterosteus aculeatus in great central lake Vancouver island, with comments on competition for food with juvenile sockeye salmon, Oncorhynchus nerka. *Fish. Bull.* 74: 647-648.
- Maynard Smith, J. (1982). Evolution and the theory of games. Cambridge University Press, Cambridge.
- Mcphail, J. D. (1977). Inherited inter-population differences in size at first reproduction in three spine sticklebacks (Gasterosteus aculeatus L.) *Hereditary* 38: 53-60.
- Milinski, M. (1979). An evolutionary stable feeding strategy in sticklebacks. *Z. Tierpsychol.*, 51: 36-40.
- Moodie, G. E. E. (1972). Predation, natural selection and adaptation in an unusual three spine stickleback. *Hereditary* 28: 155-167.
- Morris, D. (1970). Patterns of reproductive behaviour. Jonathan Cape, London.
- Mullem, P. J. van (1967). On synchronisation in the reproduction of the stickleback (Gasterosteus aculeatus L. forma leiura

- cuv.). Arch. Neerl. de Zoologie. 17: 258-274.
- Mullem, P. J. van and Vlugt, J. van der (1964). On the age, growth and migration of the anadromous stickleback Gasterosteus aculeatus L. investigated in mixed populations. Arch. neel. Zool. 16: 111-139.
- Munzing, J. (1959). Biologie, variabilitat und genetik von Gasterosteus aculeatus L. (Pisces). Untersuchungen im Elbegebiet. Int. Rev. Ges. Hydrobiol. 44: 317-382.
- Pennycuik, L. (1971a) Quantitative effects of three species of parasites on a population of three spined sticklebacks, Gasterosteus aculeatus L. J. Zool. Lond. 165: 143-162.
- Pressley, P. H. (1981). Parental effort and the evolution of nest guarding tactics in the three spine sticklebacks, Gasterosteus aculeatus L. Evolution 35(2): 282-295.
- Pugesek, B. H. (1983). The relationship between parental age and reproduction effort in the californian gull (Larus californicus). Behav. Ecol. Sociobiol. 13: 161-171.
- Rajalsilta, M. (1980). Food consumption of the three spined stickleback (Gasterosteus aculeatus L.). Ann. Zool. Fennici 17: 123-126.
- Reist, J. D. (1983). Behavioural variation in pelvic phenotypes of brook stickleback Culea inconstans in response to predation by northern pike Esox lucius. Environ. Biol. fishes. 8: 255-268.
- Reznick, D. (1983). The structure of guppy life history: The trade-off between growth and reproduction. Ecology, 64(4):862-873.
- Reznick, D. and Endler, J. A. (1982). The impact of predation on life history evolution in Trinidadian guppies (Poecilia reticulata) Evolution, 36(1): 160-177.

- Robertson, R. J. and Biermann, G. C. (1979). Parental investment strategies determined by expected benefits. *Z. Tierpsychol.* 50: 124-128.
- Rogers, D. E. (1968). A comparison of the food of sockeye salmon fry and three spine sticklebacks in the wood rivers lakes. *Univ. Wash. Publs.* 3: 1-43.
- Schaffer, W. M. and Elson, P. F. (1975). The adaptive significance of variations in life history among local populations of Atlantic salmon in North America. *Ecology*, 56: 577-590.
- Scott, D. P. (1962). Effect of food quantity on fecundity of rainbow trout, Salmo gairdneri. *J. fish Res. Bd. Can.* 19: 715-731.
- Sibly, R. and Calow, P. (1983). An integrated approach to life cycle evolution using selective landscapes. *J. theor. Biol.* 102: 527-547.
- Stanley, B. V. and Wootton R. J. (1986). Effects of ration and male density on the territoriality and nest-building of male three-spined sticklebacks (Gasterosteus aculeatus L.). *Anim. Behav.* 34, 527-535.
- Stanworth, P. (1953). A study of reproduction in the three spined sticklebacks, Gasterosteus aculeatus L. with some reference to endocrine control. Ph.D thesis, University of Sheffield.
- Staples, D. J. (1975). Production biology of an upland bully (Philypnodon breviceps Stockell) in a small New Zealand lake. 1. Life history , food, feeding and activity rhythms. *J. fish Biol.* 7: 1-24.
- Stearns, S. C. (1976). Life history tactics: A review of the ideas. *Quart. Rev. Biol.* 51: 3-47.
- Stearns, S. C. (1980). A new view of life history evolution. *Oikos* 35: 266-281.

- Swarup, H. (1958). The reproductive cycle and development of the gonads in Gasterosteus aculeatus L. Proc. Zool. Soc. 11: 47-61.
- Symons, P. E. K. (1965). Analysis of spine raising in the male three spined stickleback. Behaviour, 26: 1-74.
- Trivers, R. L. (1972). Parental investment and sexual selection. In: Sexual Selection and the Descent of man, 1871-1971. (Campbell, B. ed) Aldine Publ. Chicago, pp. 139-179.
- Tramp - Blom, N. (1959). The ovaries of Gasterosteus aculeatus L. before, during and after the reproductive period. Proc. K. ned. Akad. wet. (c) 62: 225-237.
- Wallace, R. A. and Selman, K. (1979). Physiological aspects of oogenesis in two species of sticklebacks, Gasterosteus aculeatus L. and Apeltes quadracus (Mitchill). J. fish Biol. 14: 551-564.
- Waller, D. M. and Green, D. (1981). Implications of sex for the analysis of life histories. Amer. Natur. 177: 810-813.
- Whoriskey, F.G. and FitzGerald, G. J. (1985a). The effects of bird predation on an estuarine stickleback (Pisces: Gasterosteidae) community. Can. J. Zool. 63, 301-307.
- Whoriskey, F.G. and FitzGerald, G. J. (1985b). Sex, cannibalism and sticklebacks. Behav. Ecol. Sociobiol, 18, 15-18
- Wootton, R. J. (1973a). The effect of size of food ration on egg production in the female three spined stickleback, Gasterosteus aculeatus L. J. fish Biol. 5: 89-96.
- Wootton, R. J. (1974a). The inter-spawning interval of the three spined stickleback, Gasterosteus aculeatus J. Zool. Lond. 172 331-342.

- Wootton, R. J. (1976). The biology of the sticklebacks.
Academic Press, London.
- Wootton, R. J. (1977). Effects of food limitation during the breeding season on the size, body components and egg production of female sticklebacks (Gasterosteus aculeatus) J. Anim. Ecol. 46: 823-834.
- Wootton, R. J. (1985). A functional Biology of sticklebacks. Croom Helm, London.
- Wootton, R. J. and Evans, G. W. (1976). Cost of egg production in the three spined sticklebacks, (Gasterosteus aculeatus L.) J. fish Biol. 8: 385-395.
- Wootton, R. J.; Evans, G. W. and Mills, L. (1978). Annual cycle in female three spined sticklebacks, (Gasterosteus aculeatus L.) from an upland and lowland population. J. fish Biol. 12: 331-343.

